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Roles of Chk1 in Cell Biology and Cancer Therapy

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Abstract

The evolutionally conserved DNA damage response (DDR) and cell cycle checkpoints preserve genome integrity. Central to these genome surveillance pathways is a protein kinase, Chk1. DNA damage induces activation of Chk1, which then transduces the checkpoint signal and facilitates cell cycle arrest and DNA damage repair. Significant progress has been made recently towards our understanding of Chk1 regulation and its implications in cancer etiology and therapy. Specifically, a model that involves both spatiotemporal and conformational changes of proteins has been proposed for Chk1 activation. Further, emerging evidence suggests that Chk1 does not appear to be a tumor suppressor; instead, it promotes tumor growth and may contribute to anticancer therapy resistance. Recent data from our laboratory suggest that activating, but not inhibiting, Chk1 in the absence of chemotherapy might represent an innovative approach to suppress tumor growth. These findings suggest unique regulation of Chk1 in cell biology and cancer etiology, pointing to novel strategies for targeting Chk1 in cancer therapy.

Keywords

Chk1; cell cycle checkpoints; cancer; cancer therapy

Introduction

The act of DDR and cell cycle checkpoints requires the activation of four protein kinases that form the canonical ATR-Chk1 and ATM-Chk2 pathways. While the ATM-Chk2 pathway primarily responds to DNA double-strand breaks (DSB), the ATR-Chk1 pathway recognizes a broad spectrum of DNA abnormalities ranging from UV light, to DNA replication inhibition, to virus infection, to inter-strand DNA crosslinking, and to DSB end resection¹⁻⁹.

Chk1 was initially identified by David Beach's group in 1993 as a Ser/Thr protein kinase that controls the G2/M phase transition in response to DNA damage in fission yeast¹⁰. Shortly after that, Antony Carr's group reported the identification of the same gene, named *Rad27*, in budding yeast¹¹. In 1997, Chk1 orthologs from fruit fly (*Drosophila* 'grapes'), human and mouse (*CHK1*) were identified¹²⁻¹⁵. In this review, we will summarize how Chk1 is regulated with a particular focus on human Chk1. Further, we will discuss the role of Chk1 in cancer and therapy.

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Phosphorylation and Activation of Chk1

In 1996, Rene Bernards and Nancy Walworth firstly showed that DNA damage induces Chk1 phosphorylation in *pombe*¹⁶. Similar observations were reported for human and *Xenopus* Chk1 in a caffeine-dependent manner^{13, 17}. Caffeine inhibits the activity of ATR and ATM, but not Chk1^{18, 19}, indicating that Chk1 might be a target of ATR or ATM, which preferentially phosphorylates proteins at Ser/Thr followed by Gln. Human Chk1 contains four Ser/Gln (SQ) residues (317/345/357/366). Phosphorylation at Ser-317 and 345 (or Ser-344 in *Xenopus*), and to a much lesser extent, at Ser-366, by ATR was demonstrated experimentally, but phosphorylation at Ser-357 has not been detected (Figure 1)²⁰⁻²². Importantly, such ATR-dependent phosphorylation of Chk1 is conserved from yeast to human^{23, 24}.

Phosphorylation of Chk1 requires the generation of structures containing single-stranded DNA (ssDNA) adjacent to dsDNA, in which ssDNA is coated with replication protein A (RPA) complex. A major route of ssDNA generation is likely through the uncoupling between the active helicase and stalled DNA polymerase at replication forks during S phase²⁵. This is consistent with observations that activation of Chk1 often requires the presence or activity of proteins involved in DNA replication. The ssDNA-RPA structure then functions as a platform to attract ATR and its regulatory unit ATRIP, as well as a number of regulatory factors including Rad17, TopBP1, the 9-1-1 complex, Tim/Tipin and Claspin (or scMrc1) to damage sites. Formation of this multi-subunit complex stimulates the activity of ATR, which then phosphorylates Chk1 at Ser-317 and Ser-345, leading to the activation of Chk1 and eventually the entire checkpoint pathway²⁵. Many other proteins, such as BRCA1, MCPH1 and p300/CBP, seem to participate in inducing maximal phosphorylation of Chk1 during DDR²⁶⁻³⁰.

Phosphorylation of Chk1 by ATR is important for the DDR and checkpoints. This is illustrated by studies showing that mutating Ser-345 or Ser-317 to Ala led to checkpoint defects, as well as increased sensitivity to replicative stress³¹. Interestingly, these two sites exhibit different roles in checkpoint function. Phosphorylation at Ser-317 is required for phosphorylation at Ser-345^{22, 32-34}. Yet, phosphorylation at Ser-317 alone is not sufficient for inducing maximal phosphorylation at Ser-345³⁴. The distal C-terminus of Chk1 is required for maximal phosphorylation at Ser-345 likely through providing an optimal conformation^{34, 35}. Consistent with these observations, mutation of Ser-317 to Ala in somatic cells only abrogated the G2/M phase checkpoint, whereas mutation of Ser-345 to Ala resulted in loss of both checkpoints and cell viability³³. These results led to the idea that Ser-317 phosphorylation triggers the checkpoints, whereas phosphorylation at Ser-345 is the final determinant for maximal checkpoint activation^{32, 33}.

Function of Chk1

Activated Chk1 in turn phosphorylates a number of downstream effectors to trigger a pleiotropic cellular response including transcription regulation, energy consumption alteration, cell-cycle arrest or delay, DNA repair, or cell death if the damage is too severe to repair. Here we summarize the key checkpoint functions of Chk1.

S phase DNA replication

Chk1 responds largely to genotoxic stresses in S phase (Figure 2), in which a key target is the dual-specificity phosphatase, Cdc25A. Cdc25A undergoes Chk1-dependent phosphorylation and proteasomal degradation³⁶⁻⁴⁰. As a result, the activity of Cdk2/cyclin E or Cdk2/cyclin A complex is reduced, leading to the slowing or stalling of DNA replication. Chk1 may also induce chromatin release of Cdc45, an important factor for DNA

replication⁴¹. In addition, Chk1 monitors the DNA replication during unperturbed S phase⁴². Consistently, Chk1 forms complexes with a number of proteins involved in the DNA replication machinery, including PCNA, Pol alpha and Tim/Tipin⁴³⁻⁴⁵.

A large body of evidence suggests that Chk1 regulates at least three aspects of DNA replication in S phase: (1) controlling late origin firing; (2) controlling the elongation process; and (3) maintaining stalled replication fork stability⁴⁶⁻⁵³. Accordingly, inhibition of Chk1 often leads to increased origin firing^{51, 54}. However, the overall DNA replication velocity is inhibited in Chk1-deficient cells^{50, 51, 55, 56}. As a result, cells may be stuck in the S phase with a 2N DNA content^{33, 55}. Subsequently, cells with incompletely duplicated DNA enter mitosis prematurely due to the lack of the Chk1-controlled G2/M checkpoint, undergoing a cell death process termed 'mitotic catastrophe'. The role of Chk1 in stabilizing stalled replication forks is less well understood. Chk1-inhibited cells exhibited Mus81/Eme1-dependent cleavage of stalled replication forks and the generation of DSBs⁵⁵, indicating that the DNA endonuclease, Mus81/Eme1, is involved in fork stability regulated by Chk1.

G2/M phase transition

In response to DNA damage at G2 phase, Chk1 phosphorylates and activates the Wee1 kinase, leading to phosphorylation of Cdk1 at the inhibitory Tyr-15 residue^{57, 58} (Figure 2). In addition, Chk1 phosphorylates the phosphatase, Cdc25C, at Ser-216, whose activity is required for de-phosphorylation of Cdk1 at Tyr-15 and its subsequent activation^{13, 17, 59}. This phosphorylation of Cdc25C also creates a docking site for the 14-3-3 (or pombe Rad24) family proteins¹⁴. Association with 14-3-3 leads to nuclear export of Cdc25C, preventing it from activating Cdk1/cyclin B1 in the nucleus⁶⁰. However, nuclear export of Cdc25C may be a secondary effect of Cdc25C phosphorylation⁶¹.

While Chk1 is critical for holding cells at the G2 phase in response to DNA damage, it has to be kept inactive for normal G2/M phase transition or after the damage is repaired. This can be firstly achieved through Plk1-dependent phosphorylation followed by proteasomal degradation of Claspin, the key mediator for Chk1 phosphorylation^{62, 63}. Secondly, when cells are ready for mitotic entry, Chk1 cannot be phosphorylated and activated by DNA damage. This appears to be due to the lack of recruitment of important mediator proteins required for Chk1 phosphorylation, including RPA, ATR and CtIP, to DNA damage sites⁶⁴. Interestingly, this event is controlled by PKB/AKT⁶⁴, although exactly how PKB/AKT inhibits the recruitment of these factors to DNA damage sites remains unknown. Thirdly, the rapid increase in Cdk1/Cyclin B activity at late G2 phase leads to phosphorylation of Chk1 at Ser-280/301, which somehow limits phosphorylation of Chk1 by ATR, preventing its activation by DNA damage⁶⁵. Together, these findings suggest that Chk1 activity is finely tuned to ensure a proper and timely progression of the G2/M transition.

M phase

The idea of M phase DDR is less clear than those in G1, S and G2 phases. Similarly, whether Chk1 is involved in M phase DDR is unclear, as this is a stage where ssDNA/dsDNA structures, the key element for Chk1 activation, can hardly be generated. Even though immunostaining results showed that Ser-345-phosphorylated Chk1 is detected both in normal and damaged M phase cells^{33, 66}, a potential caveat is that these phospho-antibodies recognize more than just phosphorylated Chk1 in cells.

On the other hand, increasing evidence clearly points out a role of Chk1 in normal M phase progression. When cells enter M phase, Chk1 undergoes Cdk-dependent phosphorylation (Ser-286/301)⁶⁵. This phosphorylation seems to induce the nuclear export of Chk1 to

relieve its inhibitory effect on cyclin B/Cdk1 in the nucleus, leading to mitotic progression⁶⁷. Inhibition or depletion of Chk1 leads to various mitotic abnormalities, such as chromosome misalignment on the spindle and kinetochore defects^{66, 68, 69}. Potential targets of Chk1 include the spindle assembly checkpoint proteins, Aurora A kinase⁷⁰, Plk1⁶⁸, and Aurora B kinase⁶⁹. In this regard, co-depletion of the spindle assembly checkpoint proteins rescued the M phase abnormalities caused by Chk1 depletion⁶⁸. In *Drosophila*, Chk1 (*grapes*) regulates chromosome condensation^{71, 72}, indicating conserved roles of Chk1 in M phase progression.

In mammals, a small portion of Chk1 was reported to localize to centrosomes to block premature activation of the cyclin B/Cdk1 complex, preventing abnormal M phase entry⁷³. However, recent research results showed that the anti-Chk1 antibodies used for the staining cross-reacted with another centrosomal protein⁷⁴, suggesting that the centrosomal localization of Chk1 warrants further investigation. Nevertheless, these findings clearly point out critical roles of Chk1 in M phase progression.

Other functions of Chk1

While the major function of Chk1 is to coordinate the DDR and cell cycle checkpoint response, it also regulates a number of other cellular functions, including DNA damage repair, gene transcription, embryo development and somatic cell viability^{20, 75–77}. Other protein kinases, including PKB/AKT and the MAPKAPK kinase, p90/RSK, can phosphorylate Chk1 at sites different from ATR^{78, 79}. Further, Chk1 also regulates cellular response to HIV virus infection, low or high level of oxygen exposure, protein misfolding stress, or heat shock^{8, 9, 80–83}.

Mechanisms Controlling Function and Expression of Chk1

In addition to mechanisms regulating upstream events governing the phosphorylation and activation of Chk1, recent studies from our laboratory and others illustrated a number of novel mechanisms that regulate both activation and expression of Chk1. These involve phosphorylation-coupled protein conformational change and cellular re-distribution, as well as proteasome-dependent degradation of Chk1 (Figure 3).

Conformational change of Chk1

In the absence of DNA damage, Chk1 appears to adopt a ‘closed’ conformation through an intra-molecular interaction between the N-terminus and the C-terminus^{84, 85}. This ‘closed’ conformation probably physically blocks the active site of the kinase domain of Chk1, as well as stabilizes the protein in the absence of DNA damage⁸⁵ (Figure 3). Yeast Chk1 contains a pseudosubstrate motif at the C-terminus (D469) that facilitates such intramolecular interaction⁸⁶. However, this residue is not conserved in Chk1 from other species and does not regulate the auto-inhibitory effect of Chk1⁸⁶. In response to DNA damage, Chk1 is phosphorylated by ATR on chromatin^{56, 87}. This phosphorylation disrupts the intra-molecular interaction in an unidentified manner, leading to the exposure of the catalytic domain of Chk1 (Figure 3). Since the catalytic site of Chk1 always adopts an active conformation⁸⁸, this ‘open’ conformation then unleashes the catalytic activity to turn on the checkpoint through phosphorylating downstream effector proteins. Interestingly, such auto-inhibitory regulation seems to be quite common for protein kinases, such as Src and MLCK, etc^{89, 90}.

Our recent studies revealed a new layer of function of this ‘closed’ conformation, which is to prevent Chk1 from being phosphorylated at ATR sites in the absence of DNA damage³⁴. Since Chk1 phosphorylation functions as a trigger to release the intra-molecular restraint,

preventing Chk1 from being phosphorylated by ATR is important to maintain Chk1 in the 'closed' inactive conformation under normal growth conditions. These mutual inhibitory effects between the N-terminal kinase domain and the C-terminal regulatory domain of Chk1 significantly increase the threshold of accidental checkpoint activation in the absence of DNA damage, which could be achieved either through exposing the catalytic domain or through phosphorylating the ATR sites of Chk1. Keeping Chk1 inactive under normal growth condition is critical for cell viability, as constitutive activation of Chk1 leads to cell cycle arrest and eventually cell death³⁴.

Spatiotemporal regulation of Chk1

Since Chk1 functions as the 'messenger' to deliver the DNA damage alarm, it is conceivable that Chk1 does not form stable DNA damage-induced foci. This is consistent with observations that phospho-Chk1 is expressed throughout the nucleus after DNA damage⁹¹. This phosphorylation not only induces the protein conformational change, as discussed above, but also triggers a rapid release of Chk1 from damaged chromosomal sites into the soluble nucleoplasm, and later on into the cytoplasm^{44, 56, 75, 87}. Phosphorylated Chk1 undergoes SCF^{Fbx6}- and Cul4A^{CDT2}- dependent degradation in the cytoplasm and nucleus, respectively^{56, 85, 92, 93}. Interestingly, compared to the rapid checkpoint activation, degradation of Chk1 occurs at a relatively slow rate⁵⁶, leading to around 4 hr difference between Chk1 phosphorylation and the onset of detectable Chk1 protein reduction. This may be partially determined by the intensity of DNA damage and the time required for the mobilization of phospho-Chk1 from chromatin to the nucleoplasm and eventually to the cytoplasm, where the E3 ligases are located⁸⁵. Retention of Ser-345 phospho-Chk1 in the nucleus might be facilitated through its association with 14-3-3 proteins⁹⁴⁻⁹⁶. This delayed degradation of Chk1 has significant biological implications. First, it allows active Chk1 molecules enough time to turn on the checkpoint. Second, degrading Chk1 after checkpoint activation would fine-tune the cellular checkpoint level, contributing to the ultimate checkpoint termination, cell cycle resumption and cell survival⁹⁷. While numerous studies have reported degradation of mammalian Chk1 (see review⁹⁷), yeast Chk1 does not seem to undergo degradation. One possible explanation is that the putative degron for Chk1 degradation, the CM1 motif, is highly conserved in mammals, but not in yeast⁹⁸.

The nuclear-cytoplasm shuttling of Chk1 is Crm1-dependent^{95, 99}. Chk1 does not contain a canonical NES (a Leu zipper, LxxxLxxLxL). Our recent studies suggest that the CM1 motif functions as a non-canonical NES (LxxxMxxFxxxL) that regulates nuclear export of Chk1. In contrast, the CM2 motif and flanking region contains canonical nuclear localization signals (NLS) and are responsible for nuclear localization of human Chk1, as for *Xenopus* Chk1^{84, 99}. Probably the most interesting observation of our studies is that even though the cytoplasmic pool of Chk1 is important for checkpoint function, it is the nuclear pool of Chk1 that supports cell viability⁹⁹.

Transcriptional and post-translational control of Chk1 expression

Chk1 isoforms—Mammalian *CHK1* mainly transcribes as isoform 1, which encodes the full-length Chk1 protein. However, short Chk1 isoforms exist due to alternative splicing or translation start sites or protein cleavage¹⁰⁰⁻¹⁰⁴. A question remaining unanswered is whether and how Chk1 isoforms affect cellular checkpoint function. Given the fact that the N- and C-termini interact each other, it is tempting to speculate that Chk1 isoforms or short fragments might interfere with the function of endogenous Chk1 through competitively

interacting with endogenous Chk1 molecules, although overexpressing the N-terminal kinase domain of Chk1 did not activate checkpoints in yeast³⁵.

The full-length transcript of human CHK1—Expression of mammalian Chk1 isoform 1 (both mRNA and protein) peaks at S and G2 phases of the cell division cycle¹⁰⁵, in line with its major roles in regulating DDR and checkpoints in these cell cycle phases^{34, 64}. The *CHK1* gene appears to be a target of the E2F family of transcription factors¹⁰⁶, which controls the rise of Chk1 at the G1/S transition. Therefore, stresses that can activate p53-dependent G1/S arrest often lead to reduced Chk1 expression through inhibiting E2F-dependent transcription of *CHK1*¹⁰⁷. However, the reduction in the protein level of Chk1 under both normal cell cycle transition and genotoxic stress conditions is mainly controlled by proteasome-dependent degradation⁹⁷. The de-ubiquitination enzyme, USP1, on the other hand, is involved in de-ubiquitination and stabilization of Chk1¹⁰⁸. Interestingly, nutrient restriction (e.g., glucose deprivation or hypoxia), cytokine treatment, heat shock or histone deacetyltransferase inhibition, or altering the expression of Chk1-interacting proteins also triggered proteasome-dependent degradation of Chk1^{26, 56, 109–116}, leading to up to 90% decrease in the level of Chk1.

Why are Chk1 protein levels affected by such a wide range of stresses and physiological changes? This is probably because the expression level of Chk1 is critical for its function, especially for cell viability maintenance and tissue development. Even a 50% reduction in Chk1, caused by gene disruption or chemical inhibition^{56, 68, 117–119}, significantly increased spontaneous cell death and development defects. Loss of one copy of *CHK1* leads to anemia and erythropoiesis defects and sudden death in mice¹²⁰. This suggests that Chk1 haplo-insufficiency compromises cell viability. How exactly a reduction in the level of Chk1 leads to such defects is currently unknown. It is tempting to speculate that the overall reduction in Chk1 kinase activity due to decreased Chk1 protein levels is responsible for such defects. In line with this hypothesis, a hypomorphic R156Q mutation in the kinase domain of mouse *Chk1* that reduces its catalytic activity but not protein levels, failed to support normal cell proliferation and also leads to anemia and erythropoiesis abnormalities (personal communication, Yolanda Sanchez). Together, these data indicate that Chk1 protein expression is tightly controlled both during normal growth conditions and under stressful situations.

Known Chk1 Targets

A number of studies had intended to identify Chk1 substrates using either *in vitro* peptide screening or *in vivo* cell labeling^{121–123}. An overall consensus motif for Chk1 substrate phosphorylation is R/K-R/K-d/e-t-S/T-X-r/k-r, in which upper and lower case letters represent preferred and non-preferred residues, respectively¹²³. Chk1 substrates that have been confirmed in cell cultures are summarized in Table 1.

Chk1-interacting Proteins

Chk1 exerts its function often through interacting with other proteins. Numerous proteins have been reported to interact with Chk1 (Figure 1 & Table 2), although not all of them have been reported to have biological significance. Some of these interacting proteins are also Chk1 substrates.

Chk1 in Cancer

Human *CHK1* is located in 11q22–23, a region that not only contains the highly mutated gene *ATM*, but also has frequent deletions and loss of heterozygosity in human tumors¹⁵. Given its critical roles in DDR and cell cycle checkpoints, Chk1 was initially thought to

function as a tumor suppressor, and numerous efforts were made to look for *CHK1* mutations in human tumors. However, so far no homozygous loss-of-function mutation of *CHK1* has been detected in a wide range of human tumors^{164–169}. These results suggest that a homozygous mutation that affects *CHK1* function is not able to support tumor clone expansion. Thus, those tumor clones might have died out before being detected. Since the expression level is as important as Chk1's catalytic function, an extension of this speculation is that mutations identified so far do not compromise Chk1's expression below a critical level.

Based on these observations, we hypothesize that Chk1 is unlikely a canonical tumor suppressor. Mice studies supported such a notion. Conditional knockout studies in mouse thymus and mammary gland indicate that Chk1 depletion does not increase spontaneous tumor incidence^{68, 69, 117, 118}. In fact, loss of *CHK1* reduces tumorigenicity in mice driven by *TP53*-null mutation or carcinogen exposure^{170, 171}. This is similar to the suppression of mouse skin tumorigenesis when the catalytic activity of ATR is ablated in a *XPC*^{-/-} background¹⁷². These findings suggest that Chk1 (or the ATR-Chk1 axis) is a weak tumor suppressor at best. Instead, increasing evidence suggests that the ATR-Chk1 axis, or at least Chk1, may actually promote tumor growth.

Roles of Chk1 in Tumor Etiology and Therapy Resistance

Chk1 has been found to be overexpressed in a variety of human tumors, including breast, colon, liver, gastric, nasopharyngeal carcinoma, etc.^{150, 173–178}. Remarkably, its expression often positively correlates with tumor grade and disease recurrence^{150, 176, 179}. A transgenic mouse line carrying an extra copy of *CHK1* facilitates cell transformation probably due to the enhanced ability of those cells to deal with replicative stress¹⁸⁰. These observations are well in line with the idea that Chk1 promotes tumor growth.

Further, Chk1 may also contribute to therapy resistance. Enhanced activation of Chk1 led to resistance of cancer cells, including cancer stem cells from brain glioblastoma, prostate and lung NSCLC, to chemotherapy or radiotherapy, as well as to other anticancer therapies, for instance, HDAC inhibitors^{85, 181–186}. Conversely, inhibiting Chk1 by RNAi or small molecules reversed such therapy resistance. Further, elevated levels of Ser-345 phosphorylated Chk1 proteins correlated with increased radio-resistance in metastatic brain and lung cancer patients¹⁸⁷. In addition, cancer cells may acquire chemotherapy resistance through increased expression of Chk1¹⁸⁸. Together with the fact that Chk1 is essential for the maintenance of cell viability, these findings suggest a tumor-promoting model of Chk1. In this model, tumor cells that have increased Chk1 expression possess survival advantages over their neighbors, because the more Chk1 protein cells have, the better able they are to handle the DNA damage stress caused either by the harsh tumor microenvironment (e.g., replicative stress¹⁸⁰) or by chemotherapy/radiotherapy. Eventually, these Chk1-proficient cells will grow out and dominate within the tumors, fueling the generation of more malignant, drug-resistant clones to give rise to tumor recurrence and disease relapse in the clinic. This model provides strong support to target Chk1 in human cancer therapy.

Targeting Chk1 in Human Diseases

Chemotherapy and radiotherapy kill proliferating cancer cells through generating massive DNA lesions. In the meantime, they activate the Chk1-dependent DDR and cell cycle checkpoints to facilitate cell survival. A conventional idea is that when Chk1 is inhibited, cancer cells lose their ability to respond to and repair DNA damage, enhancing the cell killing effect of chemotherapy or radiotherapy. Therefore, combining Chk1 inhibition with chemotherapy or radiotherapy provides the so-called 'synthetic lethality' effect in cancer

therapy (Figure 4). Consistent with this idea, a number of siRNA screening studies identified Chk1 as a target, which when depleted by siRNA, led to the most significantly enhanced cell killing effect among kinases by chemotherapy or radiation therapy in ovarian, triple negative breast and brain cancers^{189–191}. Furthermore, since a large portion of tumors lost the p53-dependent G1 phase checkpoint, they rely much more heavily on the Chk1-dependent S and G2/M checkpoints for survival. Therefore, Chk1 inhibition should be especially effective against p53-deficient cancer cells compared to p53-proficient cancer cells¹⁹². Recent work using a humanized mouse model of triple negative breast cancer confirmed such an idea¹⁹³.

Numerous attempts have been made by various pharmaceutical companies to identify specific Chk1 inhibitors to enhance the effect of chemotherapy^{194–196}. However, a major issue with this conventional strategy is the off-target effects and toxicity associated with Chk1 inhibitors, chemotherapy or a combination of them. So far, no therapy has reached the bedside even though theoretically a highly selective Chk1 inhibitor would synergize with chemotherapy and have a therapeutic window in combination with a DNA damaging agent (refer reviews^{194, 195, 197}). In addition, loss of *CHK1* caused developmental defects of normal blood system¹²⁰. Therefore, alternative strategies need to be developed to target Chk1 in cancer therapy.

Recently we discovered that disrupting the ‘closed’ conformation of Chk1 (e.g., by mutating one of two absolutely conserved residues G448 or L449 at the CM2 motif) leads to activation of Chk1 in the absence of DNA damage³⁴. Remarkably, expression of this constitutively active Chk1 mutant completely blocked cancer cell proliferation and eventually led to cell death under normal growth conditions³⁴. This is probably because the mutant sounds a significantly high ‘false’ alarm signal as if the cell had massive DNA damage beyond its capability to repair. These results lead to the novel concept that too much activation of Chk1 is detrimental to cell survival in the absence of DNA damage. This in turn indicates that artificially activating, but not inhibiting, Chk1 in the absence of DNA damage could be developed into an innovative strategy in cancer therapy (Figure 4). This novel strategy does not involve the use of toxic chemotherapeutic drugs; therefore, it has the potential to significantly reduce the side effect of anticancer therapy compared with conventional strategies.

Conclusion

Significant progress has been made during the last decade in understanding Chk1 regulation and its potential as a cancer therapy target. Meanwhile, many new questions have been raised. To name a few important ones: (1) How exactly does Chk1 support cell viability? Is this related solely to its checkpoint function or an as yet to be unidentified role of Chk1? (2) What are specific targets of Chk1 in the nucleus versus in the cytoplasm? Given the fact that cytoplasmic Chk1 does not support cell viability, it is important to answer this question in the near future. (3) How exactly does phosphorylation at Ser-317/345 by ATR lead to Chk1 activation? A crystal structure of the full-length Chk1 protein is clearly the key for answering this critical question. (4) How can one develop a more specific targeting strategy towards Chk1 in cancer therapy? We propose that artificially activating, but not inhibiting, Chk1 under normal growth conditions might represent a novel idea in suppressing tumor growth. In conclusion, exciting results regarding Chk1 regulation and targeting are expected to continuously emerge in the near future.

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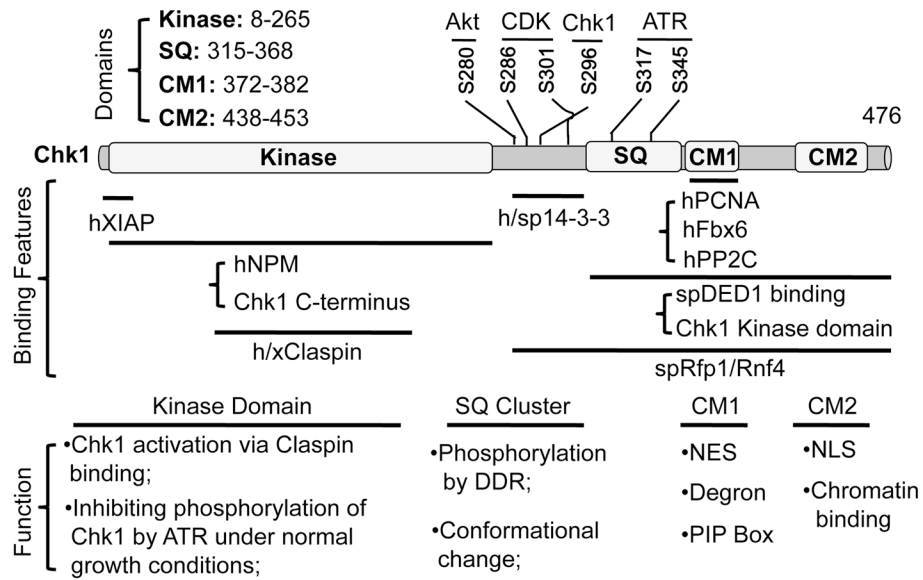


Figure 1. Human Chk1 domain structure, interacting proteins and function of each domain SQ, Ser/Gln cluster; CM, conserved motif; NES, nuclear export signal; NLS, nuclear localization signal; h, human; sp, *S pombe*; x, *Xenopus*; PIP, PCNA-interacting protein. Location of each domain is based on human Chk1. Lines illustrate regions of Chk1 that interact with other proteins. Phosphorylation sites are indicated.

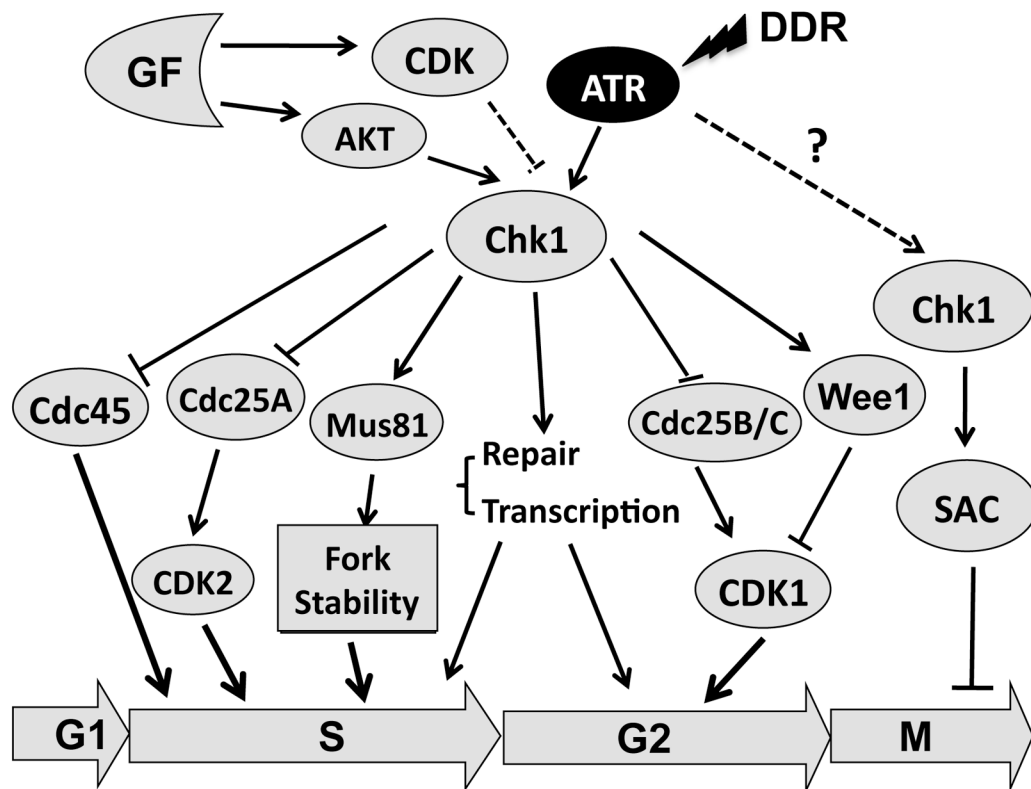


Figure 2. Cell cycle checkpoint function of Chk1.

Chk1 regulates DDR and cell cycle checkpoints during the S, G2 and M phases of the cell cycle. Growth factors (GF) also regulate Chk1. Dash lines represent as-yet confirmed signaling. SAC, spindle assembly checkpoint. See text for detail.

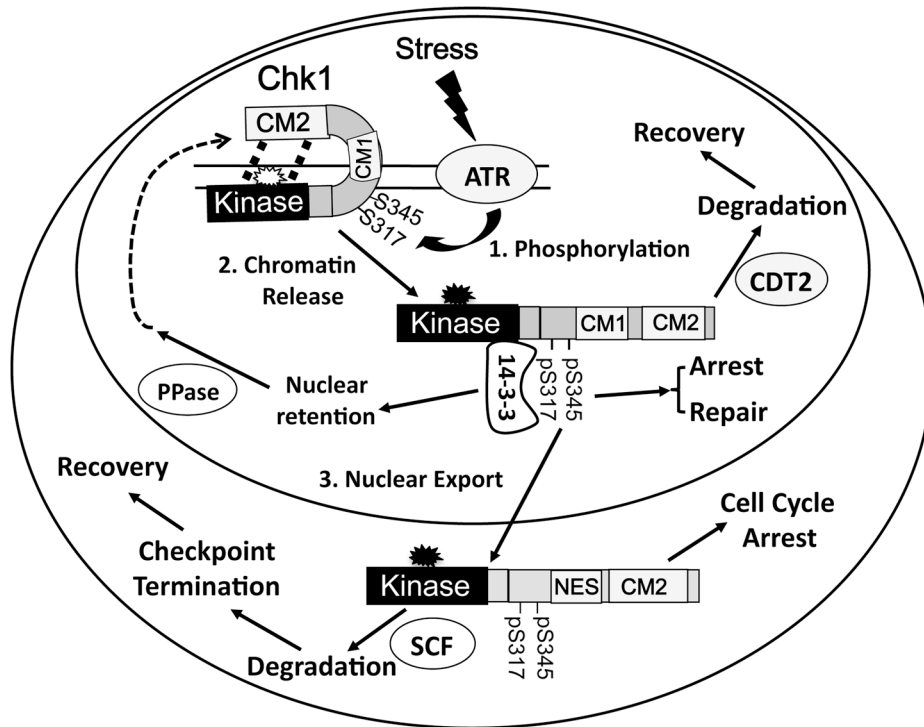


Figure 3. Spatiotemporal and conformational regulation of Chk1

The intra-molecular interaction between the N-terminal kinase domain and the C-terminal domain requires structural studies to confirm. Thus, it is shown as dash lines here. For simplicity, proteins and protein complexes involved in the signaling pathway, such as RPA, TopBP1, Tim/Tipin, are omitted in this model. The catalytic site is illustrated in open and solid stars for 'closed' and 'open' conformation, respectively. The inner and outer circles represent the nucleus and the cytoplasm, respectively. The CM1 motif bears non-canonical NES activity, facilitating nuclear export of Chk1.

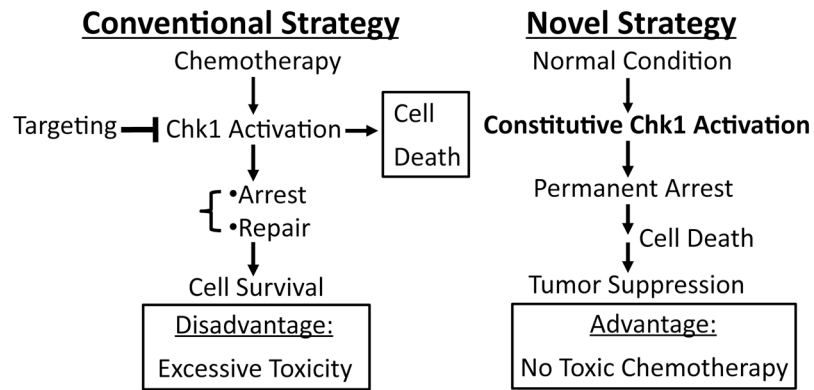


Figure 4. Strategies for targeting Chk1 in cancer therapy

Under conventional idea, a Chk1 inhibitor is combined with chemotherapy to enhance the therapy effect. In the novel approach derived from our latest research results, constitutively activating Chk1 under normal growth condition is sufficient to induce permanent cell cycle arrest and cell death. The new approach should significantly reduce the toxicity of chemotherapy since it does not require concurrent use of chemotherapy.

Table 1

Chk1 substrates, including the phosphorylation site and potential checkpoint functions

Substrate	P. Site	Substrate Regulation	Function	Ref.
Cdc25A	S76/123	Degradation	S phase arrest	37
	T507(X504)	14-3-3 binding	S & G2 arrest	127, 128
Cdc25B	S230/563	Reduced activity	G2/M arrest	129
Cdc25C	S216	Cytoplasm location	G2/M arrest	13, 14, 60
p53	S20	Stabilization	G1/S or G2/M arrest	124, 125
14-3-3 γ	S367	P53 stabilization	G1/S arrest by UV	143
DDR & Checkpoints	Nek11	Phosphorylates Cdc25A	Cdc25A degradation	130
	spPds1	Stabilization	Inhibiting anaphase	131, 132
XWee1	S549	14-3-3 binding, Cdk1pY15	G2/M arrest	133
Ttk	S695	Chromatin assembly	S checkpoints	135
KAP1	S473	Heterochromatin binding	Unclear	123, 134
Chk1	S296	Autophosphorylation	Checkpoint marker	146, 147
Aurora B	S331	Aurora B activation	Kinetochore attachment	69, 149
Rad51	T309	Unclear	Homologous repair	77
BLM	S646	Stabilization	Damage repair	138, 139
Repair	FANCE	T346/S374	Damage repair	144
	FANCD2	S331	Mono-ubiquitination	145
Metnase	S495	DNA binding	Damage repair	140
p73	S47	Trans-activation	Cell death	136
H3	T11	GCN5 recruitment	Transcription	75
p53	S366/T387	Reduced Acetylation	Transcription repression	126
p65	T505	Unclear	Transcription repression	141
p50	S329	Inhibition of DNA binding	Transcription repression	142
ING1b	S126	Stabilization	CycB1 transcription	148
p57	S19	Unclear	Preventing differentiation	151
p21	T140/S141	Unclear	Preventing differentiation	151

Table 2

Chk1 interacting proteins

Interacting Proteins	Method	Requirement	Function	Ref.
Claspin	CO-IP	Phosphorylation by CK1 γ 1	Checkpoints	152, 153
XIAP	CO-IP	Metaphase	Unknown	154
BAD	CO-IP	Unknown	Unknown	155
Timeless	CO-IP	G1/S arrest by UV	G1/S arrest by UV	43
PCNA	MS/CO-IP	Constitutive	Checkpoints	44
Polo	CO-IP	Constitutive	Checkpoints	45
Wip1	CO-IP	Constitutive	Checkpoint Off	157
MCPHI	CO-IP	Constitutive	Unclear	158
DNMT1	CO-IP	Constitutive	Chk1 cell localization	159
Fbx6	CO-IP	Phosphorylation	Chk1 degradation	85
Cul4A/DDB1	MS/CO-IP	Phosphorylation	Chk1 degradation	56, 85, 93
NPM	CO-IP	Constitutive	NPM Chromatin loading	160
MutSa	CO-IP	Constitutive	Chromatin loading	161
SpRfp1/Raf4	Y2H	No CO-IP to confirm	Damage repair	162
Fem1b	Y2H	Constitutive	Checkpoints	163