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# Deep Insight Section

# Cell cycle, checkpoints and cancer

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**Abstract:** Deep insight on cell cycle, checkpoints and cáncer.

## Introduction

Maintenance of genomic integrity is a pre-requisite for a safe and long lasting life and prevents development of diseases associated with genomic instability such as cancer. DNA is constantly subjected and damaged by a large variety of chemical and physical agents, thus cells had to set up a number of surveillance mechanisms that constantly monitor the DNA integrity and the cell cycle progression and in the presence of any type of DNA damage activate pathways that lead to cell cycle checkpoints, DNA repair, apoptosis and transcription. In recent years checkpoint pathways have been elucidated as an integral part of the DNA damage response and in fact dysfunctions or mutations of these pathways are important in the pathogenesis of malignant tumors. Understanding the molecular mechanisms regulating the cell cycle progression and checkpoints and how these processes are altered in malignant cells may be crucial to better define the events behind such a complex and devastating desease like cancer (Poehlmann and Roessner, 2010; Vermeulen et al., 2003; Aarts et al., 2013; Kastan and Bartek, 2004).

## Cell cycle regulation

The cell cycle is a succession of very well organized molecular events that give the ability to the cell to produce the exact itself's copy. The DNA replication and the segregation of replicated chromosomes are the main events of the cell cycle. The DNA replication occurs during the so called S phase (synthetic phase) which is preceded by the DNA synthesis preparatory phase (Gap1 or G1 phase), whereas the nuclear division occurs in mitosis (M phase) and is preceded by the mitotic preparatory phase (gap 2 or G2 phase). The G1, S and G2 phases represent the interphase of a proliferating cell and constitute the time lapse between two consecutive mitoses. The differentiated cells that do not proliferate enter in the so called G0 phase which is a steady state phase or resting phase (Vermeulen et al., 2003).

The progression of a cell through the cell cycle is strictly regulated by key regulatory proteins called CDK (cyclin dependent kinase) which avoid the initiation of a cell cycle phase before the completion of the preceding one. The cdks are a family of serine/threonine protein kinases that are activated at specific points of the cell cycle consisting of a catalytic subunit with a low intrinsic enzymatic activity and of a fundamental positive regulatory subunit called cyclin (Pavletich, 1999). Cyclin protein levels rise and fall during the cell cycle, activating the corresponding cdk, whereas the cdk protein levels are kept constant throughout the cell cycle. Once the complex cdk-cyclin is formed, it gets activated by the protein CAK (cdk activating protein) which phosphorylates the complex ensuring the subsequent phosphorylation of target gene products required for the progression of the cell through the cell cycle (Morgan, 1995). When quiescent cells are stimulated by mitogen signals, CDK4 and CDK6 are activated by association with D type cyclins. These above cited cdk-cyclin complexes are important for the progression through the G1 phase and the restriction point preparing the cell to the replicative phase by phosphorylating the oncosuppressor protein pRb which causes the activation of the E2F family transcription factors. The activation of CDK4 and CDK6 is followed by the subsequent activation of CDK2 by cyclin E and cyclin A, which in turn initiates DNA replication. As the DNA replication process

finishes, the Cdk1/cyclin B complex is activated leading to mitosis (Vermeulen et al., 2003; Sherr and Roberts, 1999). Until the end of G2 phase, CDK1 is phos-phorylated at Thr14 and Tyr15 by the kinases WEE1 and MYT1, resulting in inhibition of cyclin B-CDK1 activity. Mitotic entry is ultimately initiated by depho-sphorylation of these residues by the CDC25 family of phosphatases, initiating a positive feedback loop that stimulates cyclin B-CDK1 activity and entry into mitosis (Lindqvist et al., 2009). The activation status of the cdk-cyclin complexes is also monitored by negative regulation of the ATP binding site by phosphorylation in specific residues and subsequent reactivation by specific phosphatases which dephosphorylate the same residues. Inhibitory proteins also contribute to negatively regulate the cdks by forming either binary complexes with cdks or ternary complexes with cyclin cdk dimers (figure 1). Three distinct families of these so called cyclin dependent kinase inhibitors (CKI) can be distinguished. The first one is called INK family and is composed by four members: p15, p16, p18 and p19. They mainly regulate the G1-S transition of the cell cycle targeting to CDK4 and CDK6 by binding the cdk subunit and causing a conformational change of the kinases which become inactive precluding the cyclin binding. The second family of inhibitors is the Cip/Kip family and consists of three members: $p21^{cip1}$, $p27^{kip1}$ and $p57^{kip2}$. The components of this group negatively regulate the cdk2/cyclinA and cdk2/cyclinE complexes whereas they positively regulate the cdk4/6 cyclinD complexes by facilitating and stabilizing the association of cyclin and CDKs. The final class of inhibitors is the pRb protein family which consists of two members: p107 and p130. These proteins, better known as transcriptional inhibitors, act as potent cyclin E/A-cdk2 inhibitors by binding both to cyclin and to cdk sites (Vermeulen et al., 2003; Cobrinik, 2005).

An additional level of cdk regulation is the control of nuclear import/export which can be easily exemplified by the cyclinB1-Cdk1 complex that is kept out of the nucleus through an active nuclear export until late G2, when the nuclear exporting signals are inactivated by phosphorylation ensuring nuclear accumulation. The regulation of the Cdk1-cyclinB1 complex via cytoplasmic sequestration together with the negative regulatory phosphorylation of Cdk1 prevents premature phosphorylation of mitotic targets and the entry in mitosis (Yang et al., 1998). Other examples are the CDK inactivating kinases Wee1 and Myt1 located respectively in the nucleus and Golgi complex protecting the cells from premature mitosis and the 14-3-3 group of proteins that regulate the intracellular trafficking of different proteins such as the phosphatase Cdc25C (Peng et al., 1997). The above mentioned events are very well monitored by signaling pathways called checkpoints which constantly make sure that upstream events are successfully completed before the initiation of the next phase. It's in fact important that alterations in duplication of the DNA during S phase do not occur, to avoid the segregation of aberrant genetic material to the daughter cells hence ensuring accurate genetic information's transmission throughout cellular generations. Lack of fidelity in cell cycle processes creates a situation of genetic instability which contributes to the development of cancer desease. In cancer, the genetic control of cell division is altered resulting in a massive cell proliferation. Mutations mainly occur in two classes of genes: proto-oncogenes and tumor suppressor genes.

In normal cells the proto oncogenes products act at different levels in pathways that stimulate proper cell proliferation while the mutated proto-oncogenes or oncogenes can promote tumor growth due to uncontrolled cell proliferation. Tumor-suppressor genes normally keep cell numbers down, either by halting the cell cycle and thereby preventing cellular division or by promoting programmed cell death. When these genes are rendered non-functional through mutation, the cell becomes malignant. Defective proto-oncogenes and tumor-suppressor genes act similarly at a physiologic level: they promote the inception of cancer by increasing tumor cell number through the stimulation of cell division or the inhibition of cell death or cell cycle arrest. Uncontrolled cell proliferation which evolves in cancer can occur through mutation of proteins important at different levels of the cell cycle such as CDK, cyclins, CKI and CDK substrates. Defects in cell cycle checkpoints can also result in gene mutations, chromosome damages and aneuploidy all of which can contribute to tumorigenesis.

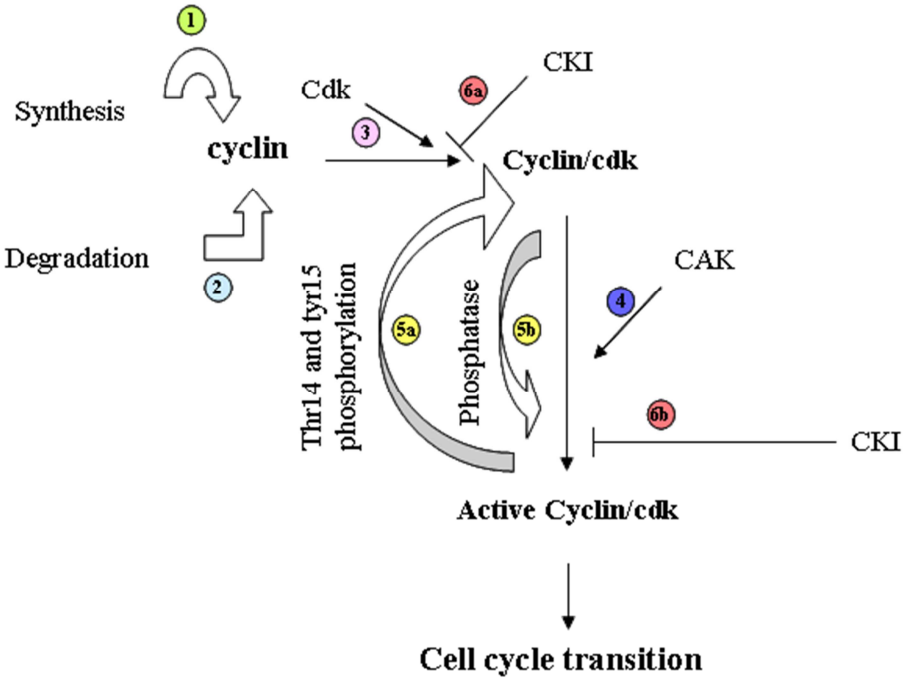


**Figure 1. Schematic summary of the levels of regulation of the cyclin dependent kinases (Cdk).** 1 and 2. Synthesis and degradation of cyclins at specific stages of the cell cycle. 3. Association of cdks to cyclins in order to be active. 4. Activation of the cdk/cyclin complexes by CAK. 5. Inactivation of cdk/cyclin complexes by phosphorylation at thr14 and tyr15 (5a) and reactivation by phosphatases acting on these sites (5b). 6. Cdk inhibitor proteins (CKI) preventing either the assembly of cdk/cyclin complexes (6a) or the activation of the cdk in the complex (6b). The activated cdk/cyclin complexes can phosphorylate substrates necessary for transition to the next cell cycle phase.

## Targeting cell cycle regulators in cancer

Cyclins and their associated cyclin-dependent kinases (CDKs) are the key drivers of the cell cycle and specific transitions in the cell cycle are controlled solely by specific CDKs. When this specificity is maintained in tumour cells, selective inhibition of these kinases presents a potential attractive strategy to tumour therapy, suggesting that a therapeutic window could be achieved. In normal cells, commitment for the progression through the cell cycle and beginning of replication process is controlled by cyclin D-CDK4/6 at the restriction point (Musgrove et al., 2011). CDK4 and CDK6 initiate the phosphorylation of the retinoblastoma (RB) protein family, resulting in dissociation and thereby activation of E2F transcription factors which initiate the S phase gene expression program, including the expression of both cyclin E and CDK2, resulting in further RB phosphorylation and ultimately S phase entry (Malumbres and Barbacid, 2009). Deregulation of the restriction point is a common event in cancer, yet CDK4/6 is a potential therapeutic target in only a subset of cancers. Many oncogenes overcome the restriction point by promoting CDK4/6 activity (Huillard et al., 2012). CDK4 can be activated more directly by point mutation/amplification or via amplification of CCND1 (cyclin D1) (Curtis et al., 2012; Kim and Diehl, 2009), or indirectly via mutation, silencing by methylation or homozygous deletion of CDKN2A (encoding p14ARF and p16INK4A) (Pinyol et al., 1997). Elevated levels of phosphorylated RB and relatively low levels of p16INK4A may provide biomarkers of CDK4/6 dependence (Konecny et al., 2011). Mouse double knockout studies of CDK4 and CDK6 suggest that the CDK4/6 kinases are only essential in specific tissue compartments (Malumbres et al., 2004), presenting a therapeutic window where tumour cells are more reliant on CDK4/6 than many proliferating normal tissues. CDK4/6 inhibition has great promise for the treatment of multiple cancer types, and multiple clinical studies are ongoing.

Cyclin B-CDK1 activity, as mentioned before, governs mitotic entry and is tightly controlled by an intricate network of feedback loops (Lindqvist et al., 2009). A number of potential issues make CDK1 a less attractive target than CDK4/6. CDK1 is essential for mitosis in most normal cells, which may limit the ability to dose CDK1 inhibitors in the clinic. If CDK1 inhibition causes a reversible G2 arrest in cancer cells, it is unclear whether a CDK1 inhibitor could be dosed sufficiently to achieve tumour control and studies are undergoing. Polo-like kinase 1 (PLK1) and Aurora kinase A (AURKA), promote progression through mitosis. Inhibition of these kinases presents a potential therapeutic opportunity through inhibiting appropriate progression through mitosis. PLK1 is a serine/threonine

kinase involved in centrosome maturation, spindle formation, chromosome segregation and cytokinesis (Strebhardt, 2010). Besides its mitotic functions, PLK1 is essential for inactivating or removing key components of the DNA damage response, such as CHK1 (via Claspin), WEE1 and 53BP1, to inactivate checkpoint signalling and promote cell cycle resumption (Strebhardt, 2010). Inhibition of PLK1 causes cells to arrest in mitosis with a monopolar or disorganised spindle followed by mitotic cell death (Lens et al., 2010). The Aurora kinase family members (A, B and C) each coordinate distinct processes during cell division. AURKA is critical for centrosome maturation and proper formation of the mitotic spindle. Selective inhibition of AURKA leads to abnormal mitotic spindles and a temporary mitotic arrest followed by chromosome segregation errors as cells exit mitosis. The amplification and overexpression of AURKA has been reported in many human tumours, including breast, colon, neuroblastoma, pancreatic and ovarian cancers, with high AURKA expression levels being associated with poor prognosis and genomic instability (Lens et al., 2010). This makes AURKA an attractive anti-mitotic drug target and as in fact, AURKA inhibitors are currently being evaluated pre-clinically and in clinical trials. Clinical data with mitotic kinase inhibitors have not yet been really promising. The AURKA-selective inhibitor MLN8237 (alisertib) had low levels of activity in a phase II study in unselected ovarian cancer (Matulonis et al., 2012), and only modest activity was seen in initial clinical trials of PLK1 inhibitors (Olmos et al., 2011). However, none of these studies have yet selected for potentially sensitive tumours, so further insights in determining the most responsive tumors are required in future trials.

## DNA damage checkpoint

A faithful transmission of genetic informations from one cell to its daughters requires the ability of a cell to survive to spontaneous and induced DNA damage to minimize the number of heritable mutations. To achieve this fidelity, cells have evolved surveillance mechanisms composed by an intricate network of checkpoint proteins that tells the cell to stop or delay the cell cycle progression providing enough time for DNA repair. When the damage could not be repaired cells undergo apoptosis. Many different lesions can occur in the cells which are coupled to different repair mechanisms. First, normal metabolic processes or exposure to external ionizing radiations generate free oxygen radicals and can break the phospho diester bonds in the backbone of the DNA helix (single strand break). When two of these breaks are close to each other but on opposite DNA strands, a double strand break (DSB) is present. Second, alkylating agents can modify purine bases and can cause intra strand or inter strand crosslinks. Inhibitors of DNA topoisomerase can cause DNA lesions leading to enhanced single or double strand
break depending on which topisomerase is inhibited and on the phase of the cell cycle. Different mechanisms are required to repair the damage to the DNA backbone or to the DNA bases and the repairing mechanisms may also vary depending on the different phases of the cell cycle.

The DNA damage checkpoint activation pathway is the response to a variety of internal factors (e.g. incomplete DNA replication due to stalled replication forks, reactive oxygen species-ROS) and external sources (e.g. UV light, ionizing radiation-IR, DNA-damaging chemotherapeutic agents).

The checkpoint activation is part of the signaling network (the DNA damage response) that involves multiple pathways including checkpoints, DNA repair, transcriptional regulation and apoptosis (Bartek and Lukas, 2007; Branzei and Foiani, 2008).

When DNA damage occurs, a signal transduction pathway cascade is activated in which sensor proteins recognize the damage and transmit signals that are amplified and propagated by adaptors/mediators to the downstream effectors that connect the checkpoint with the cell cycle machinery and final cell fate.

Generally the cell cycle progression is hampered at the stage in the cell cycle where the cell was at the time of injury: before entry in S phase (G1/S phase checkpoint), during S phase progression (intra S phase or S phase checkpoint), before mitotic entry (G2/M phase checkpoint) or during mitosis (mitotic spindle checkpoint).

The cell cycle arrest gives cell time to fix the damage by activating a series of DNA repair pathways. If the damage exceeds the capacity for repair, pathways leading to cell death are activated mostly by apoptosis (by p-53 dependent and independent pathways) (Zhou and Elledge, 2000).

Chk1 protein kinase is one of the main component of DNA damage checkpoints pathways and represent a vital link between the upstream sensors of the checkpoints (i.e. ATM and ATR) and the cell cycle engine (i.e. cdk/cyclins) (Zhou and Elledge, 2000; Stracker et al., 2009).

A brief description of its network is herein summarized to show just an example of how in general checkpoints proteins are strictly interconnected and inter-related each others.

Chk1 regulates the checkpoints by targeting the Cdc25 family of dual specificity phosphatases, Cdc25A at the G1/S and S phase checkpoints and Cdc25A and Cdc25C at the G2/M checkpoint.(Peng et al., 1997; Mailand et al., 2000)

Phosphorylation of Cdc25A by Chk1 at multiple sites increases proteosomal degradation of the phosphatase and inability of Cdc25A to interact with its cyclin/cdks substrates.

Chk1 phosphorylates Cdc25C at ser216, leading to formation af a complex with 14-3-3 proteins and cytoplasmic sequestration of the phosphatase (Peng et al., 1997; Mailand et al., 2000; Zhao et al., 2002), thus avoiding activation of the cyclinB1-CDK1 complex which regulates the entry in mitosis. Chk1 is activated after DNA damage, which ultimately causes single strand (ss) DNA breaks, by ATM- and ATR-dependent phosphorylation of C-terminal residues (ser317 and ser345). In particular, after formation of ssDNA breaks (induced for example by UV, replication stresses, DNA damaging agents), replication protein A (RPA) binds to ssDNA and recruits Rad17/9-1-1 and ATR/ATRIP complexes, leading to Chk1 phosphorylation. Chk1 activation by ATR also requires mediators such as claspin, BRCA1, TOBP1. Indirectly, as ssDNA breaks also serve as an intermediate of double strand DNA (dsDNA) breaks, ATM too is involved in Chk1 activation. ATM is recruited at the level of DSBs (induced by IR for example) by the MRN complex leading to Chk2 activation. ATM and MRN mediate DSB resection leading to ssDNA formation as an intermediate structure of DNA repair, leading to Chk1 activation through RPA/ATR-ATRIP recruitment (Bartek and Lukas, 2007; Gottifredi and Prives, 2005; Jazayeri et al., 2006).

Chk1 also plays a role in the mitotic spindle checkpoint which ensures the fidelity of mitotic segregation during mitosis, preventing chromosomal instability and aneuploidy (Carrassa et al., 2009; Zachos et al., 2007; Suijkerbuijk and Kops, 2008; Chilà et al., 2013).

## Targeting cell cycle checkpoints as therapeutic strategy in cancer

The DNA damage response requires the integration of cell cycle control via checkpoint signalling to allow time for repair to prevent DNA damage before DNA replication and mitosis take place. The importance of checkpoints pathways in the cellular response to DNA damage (both endogenous and exogenous) is at the basis of the use of checkpoint inhibitors to increase the efficacy of cancer radio- and chemo-therapy. Chemo- and radio-therapy are strong inducers of the DNA damage response pathways being able to cause different types of DNA damage and variably able to activate checkpoints, and the abrogation of these checkpoints can potentiate the cytotoxic activity of various anticancer agents (Poehlmann and Roessner, 2010). Targeting the S and G2 checkpoints has been considering attractive for cancer therapy because loss of G1 checkpoint control is a common feature of cancer cells (due to mutation of tumor suppressor protein p53), making them more reliant on the S and G2 checkpoints to prevent DNA damage triggering cell death, while normal cells also depend on a functional G1 checkpoint (Dai and Grant, 2010; Ma et al., 2011). Experimental evidence showed that inhibiting the S and G2 checkpoints by inactivation of ATR or CHK1 abrogated DNA damage-induced G2 checkpoint arrest and sensitized cancer cells to a variety of DNA-damaging chemotherapeutic agents (Carrassa et al., 2004; Ganzinelli et al., 2008; Massagué, 2004). Furthermore, oncogenic replicative stress may render cancer cells sensitive to inhibitors that prevent the S and G2 checkpoints as single agents. As mentioned previously, CHK1 is a key signalling kinase involved in the intra-S phase and G2/M checkpoints (Kastan and Bartek, 2004). In response to replication stress or genotoxic insults, CHK1 is activated via ATR-dependent phosphorylation. During unperturbed S phase, CHK1 controls replication fork speed and suppresses excess origin firing (Petermann et al., 2010), prevents premature activation of cyclin B-CDK1 and may be involved in spindle checkpoint signalling (Zachos et al., 2007; Chilà et al., 2013, Carrassa and Damia, 2011). Oncogene driven replication is abnormal and results in high levels of replication stress, and inhibition of CHK1 may increase the replication stress to sufficiently high levels to be lethal as a single agent in certain contexts (Jazayeri et al., 2006; Syljuåsen et al., 2005). The tyrosine kinase Wee1, together with Chk1, has also to be considered a crucial checkpoint protein controlling S and G2 checkpoint (Figure 2). The WEE1 kinase prevents mitotic entry via inhibitory phosphorylation of CDK1 at Tyr15 (Lindqvist et al., 2009). Recently, it is becoming clear that WEE1 is also required for the maintenance of genome integrity during DNA replication (Sørensen and Syljuåsen, 2012; Beck et al., 2012). WEE1 controls CDK1 and CDK2 activity during S phase, thereby suppressing excessive firing of replication origins, promoting homologous recombination, and preventing excessive resection of stalled replication forks (Beck et al., 2012; Krajewska et al., 2013).

Thus both Chk1 and Wee1 are required during normal S phase to avoid deleterious DNA breakage, and thereby prevent loss of genome integrity in the absence of exogenous DNA damage (Sørensen and Syljuåsen, 2012). Several Chk1 and Wee1 inhibitors have now been developed and tested in combination with DNA damaging agents to increase their efficacy, especially in tumors with a defective G1/S checkpoint (e.g. p53 defects) (Carrassa and Damia, 2011; Stathis and Oza, 2010)

WEE1 inhibitors have been developed, and some have entered into clinical trials but clinical data are still limited.

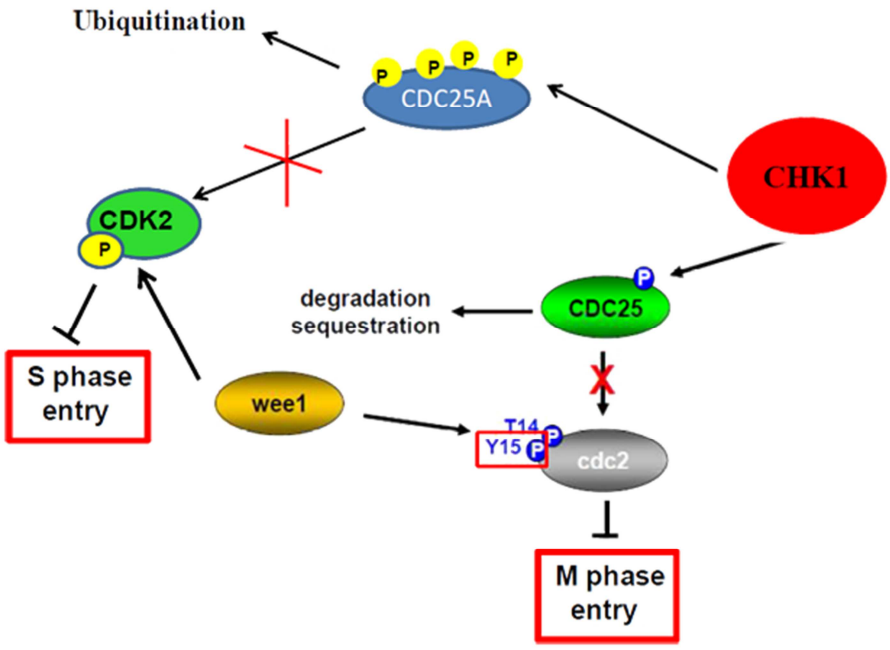


Figure 2. Schematic representation of the role of Chk1 and Wee1 in regulation of the CDK-cyclin complexes involved in S phase and M phase entry.

The pyeazolo-pyrimidine derivative MK-1775 is the most potent and highly selective inhibitor of Wee1, and has recently reached phase I (in combination with gemcitabine, cisplatin, or carboplatin) and II studies (in combination with paclitaxel and carboplatin in ovarian cancer) (Stathis and Oza, 2010; De Witt Hamer et al., 2011). Most research has focused on the development of CHK1 inhibitors, which have entered clinical studies. UCN 01 was the first of this type of inhibitor to enter clinical trials, but after Phase II trials it was discontinued owing to dose-limiting toxicities and a lack of convincing efficacy that was probably due to poor specificity and pharmacokinetics. The newer, more specific inhibitors of CHK1 have generally been combined with gemcitabine in Phase I studies, in which myelosuppression was the major toxicity that led to the termination of the trials, and no efficacy data have yet been presented (Carrassa and Damia, 2011; Blasina et al., 2008). Recently, a selective orally available inhibitor developed from a high-throughput screening hit, GNE-900, gave promising pre-clinical studies and is now undergoing Phase I clinical trials (Blackwood et al., 2013).

## Synthetic lethality approach in cancer therapy

The most promising prospect for the future of cancer treatment seems to be the exploitation of dysregulated DNA Damage Response, by the synthetic lethality approach. The synthetic lethality concept states that mutations of two different genes are not lethal in the cells when they occur at once, but are synthetically lethal, causing cells to die, if they occur simultaneously. Synthetic lethal interactions have been widely reported for loss and gain of function mutations. The synthetic lethality-driven approach offers the ideal cancer therapy as it allows indirect targeting of non-druggable cancer-promoting lesions with pharmacological inhibition of the druggable synthetic lethal interactor and as it should be exclusively selective for cancer cells, and well tolerated by healthy normal cells, that lack the cancer-specific mutation, with a wide therapeutic window (Kaelin Jr, 2005; Canaani, 2009). This concept is at the basis of the efficacy in preclinical systems of PARP inhibitors in homologous recombination defective cells, due to mutation of genes such as BRCA1/BRCA2 and it has already undergone proof-of-principle in the clinical setting. Substantial durable antitumor activity was observed after treatment with PARP inhibitors in patients with BRCA1/2-mutated cancers, including ovarian, breast and prostate cancers (Bryant et al., 2005; Fong et al., 2009). Chk1 inhibition has been proposed as a strategy for targeting FA (Fanconi Anemia) pathway deficient tumors. In fact, tumor cells deficient in the FA pathway are hypersensitive to Chk1 inhibition, suggesting a possible use of these inhibitors in FA deficient tumors (Chen et al., 2009). The FA pathway is a DNA repair pathway required for the cellular response to different DNA damaging agents, including cross-linking agents (e.g. cis-platinum) in cooperation with the homologous recombination pathway.

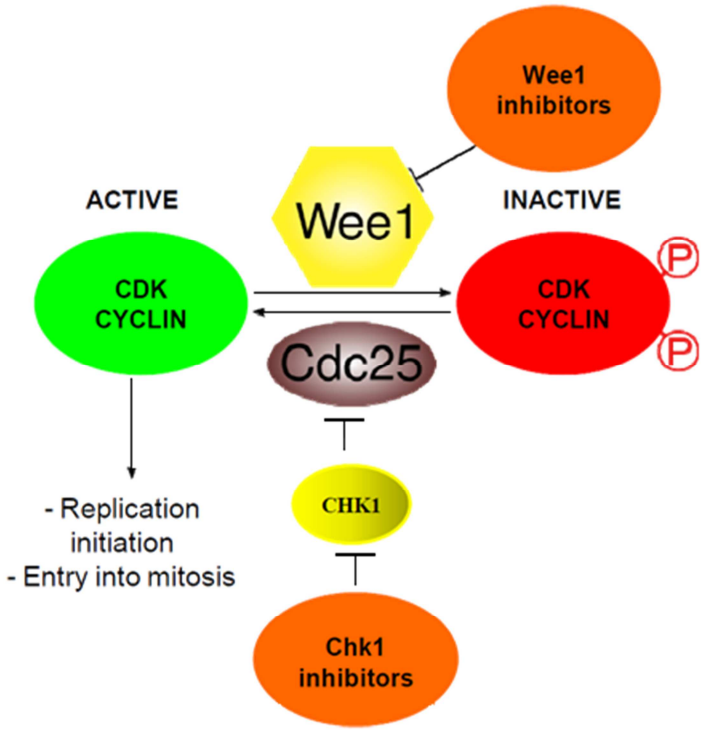


Figure 3. Schematic representation of the effects of Chk1 and Wee1 inhibition on CDK-CYCLIN complex regulation, that gets more activated being unphosphorylated.

A range of sporadic tumors with genetic and epigenetic disruption of the FA genes have been reported. Hyperactive growth factor signalling and oncogene-induced replicative stress increase DNA breakage that activates the ATR-CHK1 pathway, and some examples of the synthetic lethality of checkpoint or DNA repair inhibitors in cells harbouring activated oncogenes have been identified. ATR knockdown was synthetically lethal in cells transformed with mutant KRAS (Gilad et al., 2010), and inhibition of CHK1 and CHK2 significantly delayed disease progression of transplanted MYC-overexpressing lymphoma cells *in vivo* (Ferrao et al., 2011).

Many recent studies with a high throughput siRNA screening approach led to identification of other possible target genes synthetically lethal with Chk1 inhibitors. Recently two distinct siRNA high-throughput screening identified Wee1 as in synthetic lethality with Chk1 (Davies et al., 2011; Carrassa et al., 2012) and combined treatment of Chk1 and Wee1 inhibitors showed a strong synergistic cytotoxic effect in various human cancer cell lines (ovary, breast, prostate, colon). The strong *in vitro* synergistic effect of the combination translates to tumor growth inhibition *in vivo* (Carrassa et al., 2012; Russell et al., 2013). Simultaneous inhibition of CHK1 and WEE1 induces cell death through a general mis-coordination of the cell cycle (figure 3), which leads to DNA damage and collapsed replication forks during S phase (Carrassa et al., 2012; Guertin et al., 2012), and to premature mitosis directly from S phase. These data have been recently corroborated by other groups, suggesting that at least in solid tumors this drug combination could be a very new promising anticancer strategy deserving clinical investigation (Russell et al., 2013; Guertin et al., 2012). Many other successful synthetic lethality combinations exist and many more probably need to be explored and they will provide in the near future new potential effective tools for cancer therapy (Reinhardt et al., 2013; Curtin, 2012).

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