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SURVEY AND SUMMARY

Safeguarding genome integrity: the checkpoint kinases ATR, CHK1 and WEE1 restrain CDK activity during normal DNA replication

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ABSTRACT

Mechanisms that preserve genome integrity are highly important during the normal life cycle of human cells. Loss of genome protective mechanisms can lead to the development of diseases such as cancer. Checkpoint kinases function in the cellular surveillance pathways that help cells to cope with DNA damage. Importantly, the checkpoint kinases ATR, CHK1 and WEE1 are not only activated in response to exogenous DNA damaging agents, but are active during normal S phase progression. Here, we review recent evidence that these checkpoint kinases are critical to avoid deleterious DNA breakage during DNA replication in normal, unperturbed cell cycle. Possible mechanisms how loss of these checkpoint kinases may cause DNA damage in S phase are discussed. We propose that the majority of DNA damage is induced as a consequence of deregulated CDK activity that forces unscheduled initiation of DNA replication. This could generate structures that are cleaved by DNA endonucleases leading to the formation of DNA double-strand breaks. Finally, we discuss how these S phase effects may impact on our understanding of cancer development following disruption of these checkpoint kinases, as well as on the potential of these kinases as targets for cancer treatment.

INTRODUCTION

Maintenance of genome integrity is essential to prevent development of diseases associated with genomic instability such as cancer and neurodegenerative disorders (1). The genome integrity of human cells is frequently threatened by DNA damage caused by exogenous or endogenous sources. To counteract such threat, human cells contain a network of DNA damage surveillance pathways that maintain genome integrity. These surveillance pathways control processes such as DNA repair, cell-cycle checkpoints, apoptosis and transcription. The checkpoint kinases ATR, CHK1 and WEE1 are key regulators of DNA damage surveillance pathways. ATR and CHK1 regulate the S and G2 checkpoints (2–5), replication initiation and replication fork stability (6–10) and homologous recombination repair (11–13). CHK1 also controls mitotic entry in unperturbed cells (14) and was reported to play a role in mitotic spindle checkpoint function (15), and in control of transcription (16). WEE1 has a major cell-cycle function in control of the G2/M transition (17,18).

ATR, CHK1 and WEE1 are in fact active during unperturbed normal cell-cycle progression, and all three kinases have roles besides their functions in the response to exogenous DNA damage. Deletion of ATR, CHK1 or WEE1 in mice causes embryonic lethality (19–23), demonstrating that these checkpoint kinases are essential for embryonic development. Importantly, recent work has revealed that the activities of these kinases are required during normal S phase to avoid deleterious DNA breakage, and thereby prevent loss of genome integrity in the absence of exogenous DNA damaging agents (24–27). Here, we review the progress in this area with focus on discussing the potential mechanisms involved in causing DNA breakage following inhibition of these checkpoint kinases. We also discuss the possible impact of these findings on our understanding of the roles of these checkpoint kinases in cancer development, and on the potential of these kinases as targets for cancer treatment.

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Activation of ATR, CHK1 and WEE1

Before discussing the mechanisms how ATR, CHK1 and WEE1 are required to prevent loss of integrity in normal S phase, we briefly summarize the current knowledge about how these kinases are activated and how they are thought to control DNA replication.

Activation of ATR occurs upon the generation of lesions containing single-stranded DNA (ssDNA) (28), which evolve at stalled replication forks and following processing of DNA strand breaks (29,30). Coating of ssDNA by the single-strand binding protein RPA helps loading of ATR to DNA damage sites (31–33). ATR recognition of RPA-coated ssDNA is dependent on the ATR-interacting protein (ATRIP) (34), which binds RPA directly (35). ATR activation is also dependent on TOPBP1, RAD17 and the 9-1-1 (RAD9-RAD1-HUS1) complex (36–38). RAD17 is recruited by RPA-coated ssDNA and loads the 9-1-1 complex, which subsequently recruits TOPBP1 and brings it in close proximity to ATR so that TOPBP1 can activate ATR via direct interaction (39). Studies in *Xenopus* extracts suggest that ssDNA in itself is not sufficient to cause strong activation of ATR. While ATR activation by naked ssDNA pieces was low, high levels of ATR activation were observed at areas of ssDNA with 5′-primed ends (37). It has been suggested that these ends may be the loading site for the 9-1-1 complex (40). Thus, small pieces of ssDNA generated during normal replication do not lead to strong checkpoint activation, although they might potentially contribute to the low levels of ATR activation observed during normal unperturbed cell cycle.

CHK1 is a direct downstream target of ATR. ATR phosphorylates CHK1 on Ser 317 and Ser345 and stimulates its function (22,41,42). After the ATR induced phosphorylation, CHK1 undergoes autophosphorylation at Ser296 (43,44). The DNA damage-induced ATR phosphorylation most likely does not up-regulate CHK1 kinase activity per se. However, phosphorylated CHK1 can dissociate from chromatin (45,46), and ATR regulation of CHK1 may thereby control transition of DNA damage signals from chromatin to its targets.

The activity of WEE1 increases during S and G2 phases in parallel with its increased protein level (47), and can be stimulated by the CDK-interacting protein CABLES (48). In budding yeast, WEE1 activity is also stimulated by low levels of CDK activity in G2 phase (49). In *Xenopus*, activated XCHK1 phosphorylates the XWee1 kinase, contributing to increased Tyrosine 15 phosphorylation and inhibition of CDK activity following CHK1 activation (50). This regulatory mechanism has so far not been described in mammalian cells. At entry into mitosis WEE1 is inhibited both by phosphorylation and degradation allowing rapid increase in CDK activity (49,51–53). Subsequent CDK phosphorylation primes WEE1 for ubiquitylation via the β-TRCP SCF type of ubiquitin ligase, and this activity may be further supported by the Tome-1 SCF ubiquitin ligase (54).

The cell-cycle regulatory roles of ATR/CHK1 and WEE1 in S and G2 phases are mainly through regulation of CDK activity (Figure 1). WEE1 directly catalyses the inhibitory Tyrosine 15 phosphorylation of CDK1 and CDK2 and thereby inhibits CDK activity (18,47). Following its activation, CHK1 directly phosphorylates CDC25A (3), facilitated by 14-3-3γ (44). CHK1 activates NEK11 that also phosphorylates CDC25A directly (55), and the concerted action of the two kinases promotes the ubiquitin dependent degradation of CDC25A. The degradation of CDC25A leads to decreased removal of the phosphorylation of the CDK Tyrosine 15 residue. This results in inhibition of CDK activity that induces cell-cycle arrest (33,56). CHK1 can also phosphorylate CDC25B and CDC25C (5,57), which may also contribute to restrain CDK activity.

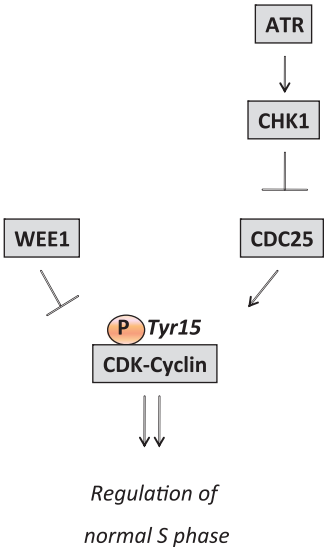

Figure 1. Regulation of CDK activity by ATR, CHK1 and WEE1 determines replication initiation during normal S phase. CDK activity is negatively regulated by phosphorylation of the Tyrosine 15 residue. This residue is phosphorylated by WEE1 and dephosphorylated by CDC25. CDC25 is negatively regulated by CHK1, which in turn is stimulated by the ATR kinase.

CDK mediated control of DNA replication initiation

When cells reach S phase, DNA replication is initiated from a large number of chromosomal locations known as replication origins. The precise activation of origins is a key part of replication control, because cells are thought to be unable to regulate the speed of the DNA polymerases. The initiation of DNA replication is triggered by the action of the CDK kinases and DBF4-CDC7 kinase (DDK) (58,59). CDK2 is considered a key CDK in control of DNA replication in S phase, although CDK1 can compensate if CDK2 is disabled (60). Once activated, CDK2 activity promotes origin firing through loading of CDC45L and AND-1/CTF4 at origins (61,62). The presence of CDC45L and AND-1/CTF4 at origins are required for origin unwinding by the MCM helicase, and thereby also required for the subsequent binding of the primase DNA polymerase (Pol) alpha that initiates DNA synthesis (61,63). Recent data suggest that the novel replication factors Treslin and GEMC1 are key

phosphorylation targets of CDK2 that promote CDC45L loading (64,65).

In addition to promoting origin firing, CDK activity also seems to regulate the activation of individual replication clusters and the overall timing of replication through S phase, although this regulation is not well understood (66–69). A substantial number of origins are fired at distinct times through S phase to allow coordinated execution of DNA replication. In fact, more origins are licensed than are ever used, and in a normal S phase most origins are replicated passively by replication forks coming from neighbouring activated origins (70). When replication fork stalling or replication stress occurs, local dormant origins will fire to compensate for the lack of replication. Under conditions of exogenous DNA damage, checkpoint pathways block the activation of origins that normally fire throughout S phase, which constitutes the basis for the S phase checkpoint (70–72).

ATR, CHK1 and WEE1 control CDK activity to support genome integrity during DNA replication

Recent studies have uncovered that ATR, CHK1 and WEE1 are critical for coordinated duplication of the genome and preventing a catastrophic outcome of the sensitive replication process. In response to CHK1 inhibition by the drugs UCN-01 or CEP-3891, or siRNA mediated depletion of CHK1, the genome is rapidly destabilized and accumulates a massive amount of DNA double-strand breaks (27). This damage is preceded by massive accumulation of ssDNA near the replication fork. All these critical downstream effects of CHK1 inhibition are dependent on CDK activity (27). The deleterious effects of CHK1 inhibition can be blocked by depleting CDC25A, the CDK activator controlled by CHK1. Furthermore, cellular fitness of CHK1 depleted cells is markedly improved by co-depleting CDC25A (26). Consistent with these results, conditional CHK1 heterozygosity causes accumulation of DNA damage during DNA replication in mice in the absence of exogenous DNA damaging agents (24). WEE1 depletion by siRNA transfection also rapidly induces DNA damage in S phase in replicating areas, which is accompanied by a marked accumulation of ssDNA. Similar to the effects of CHK1 inhibition, these deleterious S phase effects of WEE1 depletion are highly dependent on CDK activity (26).

Reduced ATR function can also cause a DNA damage response in S phase cells. Cultured MEFs and embryos from ATR mutated Seckel syndrome mice revealed high incidence of Cyclin A positive cells with strong pan-nuclear staining of γ-H2AX, consistent with increased replication stress (25). Induction of DNA damage, as assessed by increased γ-H2AX staining, was also observed in another mouse model hypomorphic for ATR (73). ATR depletion leads to deregulation of CDC25A levels in the absence of exogenous replication inhibitors and DNA damaging agents (74). In line with this, the DNA damage occurring after ATR inhibition is also blocked by depleting CDC25A, indicating that the loss of genomic integrity occurs as a consequence of deregulation of the ATR-CHK1-CDC25A-CDK pathway (75). These results suggest that similar to CHK1 and WEE1, ATR is also required to prevent formation of deleterious DNA lesions during normal S phase through control of CDK activity.

Increased CDK activity leads to unscheduled initiation of DNA replication origins

When ATR and CHK1 activity is lost, several cellular pathways are affected and could potentially contribute to the devastating cellular outcome. However, the loss of each of WEE1, ATR and CHK1 leads to increased CDK activity (3,26,74–76) resulting in a loss of control of the replication coordination. Inhibition of CHK1 leads to an increased loading of replication factor CDC45L onto chromatin, which is followed by a dramatic increase in replication initiation. Shortly after, there is increased ssDNA formation and RPA loading indicative of replication stress (27). In addition, CHK1 inhibition also causes slower replication fork speed (6,7), however, this is likely not directly due to the elevated CDK activity operating at existing forks. In fact, it was suggested that CHK1 indirectly promotes replication fork progression as a downstream consequence of the key role in control of replication initiation (7). Similarly to CHK1, WEE1 depletion causes increased ssDNA and RPA loading indicative of replication stress (26). Drug-based WEE1 inhibition also leads to a very rapid increase in origin firing and slower replication fork speeds (our unpublished observations). Importantly, DNA damage arising after both CHK1 and WEE1 inhibition, respectively, is ablated by partial suppression of DNA replication initiation obtained by depletion of CDC45L, CDT1 and MCM complex members (26,27). Based on our previous data and the common regulation of CDK activity by ATR/CHK1 and WEE1 kinases, we propose that the major cellular defect leading to DNA breakage in S phase following depletion of these kinases is CDK-driven unscheduled initiation of a large number of origins (Figure 2). Supporting the notion that CDK-driven events can lead to DNA breakage in S phase, other ways of enhancing CDK activity such as overexpression of Cyclin E, CDC25A or E2F1 also causes DNA damage in S phase (77). Interestingly, this chain of events has not been observed in yeast. Elevated CDK activity can induce chromosomal rearrangements in budding yeast by mechanisms rather involving inhibition of DNA replication licensing. However, these effects occurred only in G1 phase cells (78), and are therefore different from the S phase effects observed in human cells.

Unscheduled initiation is required for the generation of DNA lesions at the replication fork

Although unscheduled initiation is involved in the massive induction of DNA breaks after checkpoint kinase inhibition, it is not clear how it specifically contributes to break formation. A low but significant number of DNA breaks may be induced in several ways by the replication process itself (79). Importantly, unwound, single-stranded DNA at replication forks may be more susceptible to DNA breakage. This could for example occur after free radical

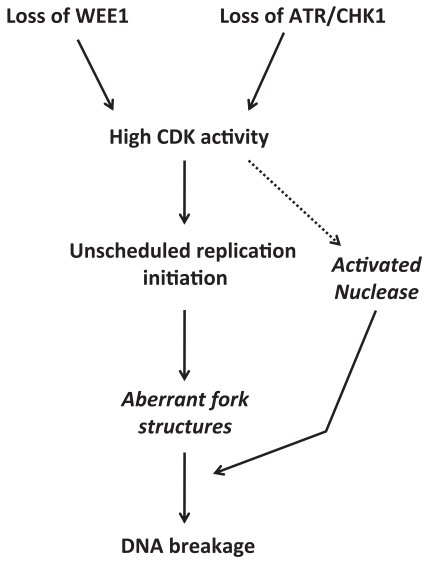

Figure 2. Model describing potential mechanisms how loss of checkpoint kinases may cause DNA breakage during normal S phase. Loss of WEE1, ATR or CHK1 leads to increased CDK activity, which causes unscheduled replication initiation and subsequent DNA breakage. Following massive unscheduled initiation of replication, aberrant fork structures accumulate that are prone to breakage resulting in DNA DSBs. The high CDK activity may activate nucleases that directly catalyse the formation of DNA DSBs.

attacks on the DNA backbone; however, checkpoint kinase inhibition is not expected to rapidly induce high levels of free radicals. In addition, ssDNA breaks existing in front of the replication fork in the template DNA can be converted to a double-strand break. During replication, the template strands on each arm of replication forks will no longer be base-paired to their original complementary template strands due to the actions of DNA helicases. Consequently, single-strand lesions within template DNA can cause double-strand breaks when the replication fork reaches such lesions. Thus increased initiation of DNA replication may in itself be sufficient to cause small amounts of DNA breakage if single-strand lesions are present. However, additional events are most likely required to achieve the massive induction of DNA breakage observed following CHK1 or WEE1 inhibition (26,27).

In response to CHK1 inhibition, replication initiation is first increased, followed by subsequent fork stalling (6,7,27). ATR and CHK1 are known to directly support fork stability (79,80). It is therefore possible that lack of ATR/CHK1-mediated support of stalled forks will lead to fork collapse and DNA breaks. Data from budding yeast have clearly documented an important role for ATR (Mec1) and CHK1 (Rad53) in fork stability, however, this function is observed under conditions of massive fork stalling with hydroxyurea, an inhibitor of nucleotide metabolism (81,82). It is not clear if fork destabilization is an important contributor to the loss of genomic integrity after checkpoint kinase inhibition under unperturbed conditions in mammalian cells. Furthermore, it is important to note that the genome integrity protection mediated by CHK1 operates largely through control of the CDC25A-CDK pathway. Similarly, WEE1 also controls genome integrity via suppression of CDK activity (26). Given these observations, it is apparent that elevated CDK activity should destabilize existing replication forks if this was the major mechanism. It remains to be shown if this is the case. In addition, the massive unscheduled initiation could perhaps result in unbalanced or insufficient nucleotide pools that could potentially lead to fork stalling and eventual collapse, similar to that observed in cells treated with inhibitors of nucleotide metabolism such as hydroxyurea (83). However, this would likely not occur with the rapid kinetics observed after CHK1 and WEE1 inhibition, where inhibitors induce DNA damage within 2 h of treatment. In contrast, DNA damage upon hydroxyurea treatment alone is normally only observed after ~24 h of treatment (84).

Checkpoint kinases or CDK may directly regulate nucleases

In addition to the unscheduled initiation of origins, additional possible explanations may more directly explain the occurrence of double-strand breaks after checkpoint kinase inhibition. It appears unlikely that such massive DSB accumulation can arise without the activity of enzymes that break the DNA backbone, which is a very solid structure. Stalled replication forks with exposed ssDNA are processed by a number of enzymes to promote genomic integrity. Homologous recombination pathways can salvage stalled forks (85), and the remodelling of the replication fork can generate structures that are resolved by endonucleases such as MUS81/EME1. The resulting double-strand break can be used to initiate fork restart (86). However, the DSBs may have additional roles since it can amplify checkpoint signalling to avoid premature mitotic entry, and if present in large amount they may serve to trigger cell death or senescence.

Nuclease substrate structures such as Holliday junctions could potentially be formed due to elevated activity of helicases that act upon stalled replication forks. Following ATR/CHK1 and WEE1 inhibition, increased CDK activity initiates excess origin firing, and this may lead to fork stalling due to shortage of important factors such as nucleotides or key replication proteins (Figure 3). The formation of nuclease substrates may also be promoted by high level of torsional stress, potentially occurring due to the elevated replicative helicase activities at origins. Torsional stress in front of the fork is thought to be able to promote reversal of the fork, since stress relief can occur via fork reversal (Figure 3). Massive fork reversal could potentially promote nuclease cleavage of the Holliday junctions and thereby cause high number of DNA double-strand breaks. Given that checkpoint inhibition leads to a large number of DNA replication fork lesions, it is likely that excellent substrates for such nucleases will appear in large quantity. The number of breaks following nuclease activity may overwhelm the cellular capacity to repair and process these lesions. The presence of breaks is further problematic to cells as CHK1 inhibition suppresses the HR pathway that would normally process the breaks (11).

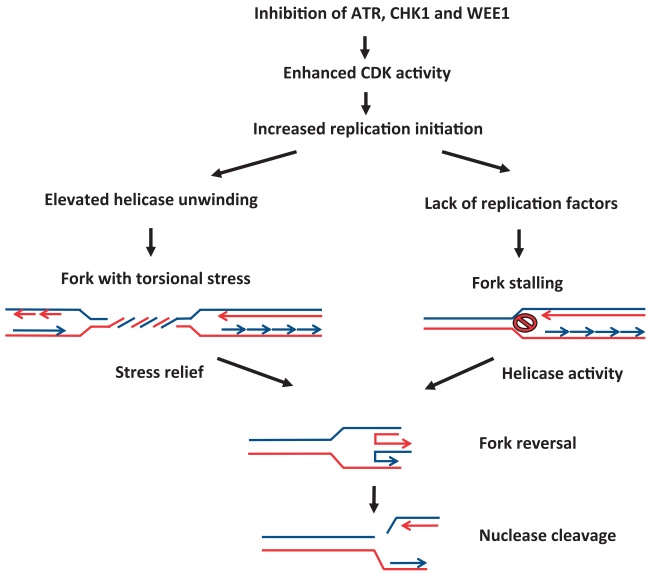

Figure 3. Model depicting how inhibition of checkpoint kinases may lead to double-strand breaks after replication fork reversal. When checkpoint kinases are inhibited CDK activity is up-regulated leading to increased origin firing. As a consequence of the enhanced origin activity, replication factors such as polymerase subunits may become limiting leading to fork stalling. Helicases can process stalled forks leading to fork reversal, which generates substrates for DNA endonucleases such as MUS81. Fork reversal may also occur due to increased initiation of replication that generates enhanced torsional stress due to helicase unwinding of the double-stranded DNA. This stress can be relieved by fork reversal, thereby forming substrates for endonuclease cleavage.

In fission yeast, it was shown that the CHK1 limits the effects of the endonuclease MUS81/EME1 at stalled forks (87). If CHK1 negatively regulates such endonucleases during normal DNA replication, loss of CHK1 could cause activation of the endonucleases and thereby lead to massive induction of DNA breaks. On the other hand, given that WEE1 depletion leads to a similar phenotype, it would rather be expected that increased CDK activity is the causative effect in activating such a DNA processing activity. Hence, it is possible that CDK has direct targets with enzymatic activity, i.e. a nuclease exhibiting aberrant activity, which could cause the massive induction of DNA damage (Figure 2). It remains to be determined to what extent such activities control genome integrity as well as the nature of the deregulated enzymes. Such nucleases should operate on the DNA structures generated by the excess CDK activity, and the elucidation of the contributions of such factors to genome integrity is an important task for future research.

DNA damage arising in S phase after acute inhibition of WEE1, ATR and CHK1 is likely dependent on nuclease activities. However, more subtle degrees of inhibition of these checkpoint kinases, as may more commonly occur *in vivo*, does not necessarily depend on nucleases. Fragile sites are areas in the genome that are prone to breakage after ATR and CHK1 inhibition, and recent evidence suggest that fragile sites can break in mitosis when sister chromatids are separating in anaphase (88). These sites are areas with few origins of replication, that critically depend on functional origins of DNA replication since neighbouring origins cannot compensate if forks stall and collapse (89). The dysfunctional replication upon reduced WEE1, ATR and CHK1 activity could lead to cells progressing into mitosis with unreplicated DNA. This is likely enhanced by G2 checkpoint deficiency upon reduced activity of these kinases. The outcome would be sister chromatid connections when chromosomes separate in anaphase, where breaks can occur by mechanical forces although nucleases may also cleave these structures.

ATR, CHK1 and WEE1 can affect malignant transformation

An important issue is whether loss of genomic integrity arising from deregulated replication caused by ATR, CHK1 and WEE1 disruption may contribute to cancer development. Heterozygous ATR and CHK1 mutations have been found in a subset of endometrial, colon, melanoma and stomach cancers (90–96). Several reports suggest that WEE1 function may also be compromised in cancer. Recently, it was shown that microRNA-155 (miR-155), which is frequently elevated in human cancers, cause down-regulation of WEE1 (97). Moreover, prostate epithelium, which is prone to prostate cancer development, expressed very low levels of WEE1 (98).

Studies of ATR and CHK1 heterozogosity in mice also suggest that compromised expression of these checkpoint kinases might contribute to cancer development. One report describes a modest increase in late tumour development in ATR heterozygous (+/−) mice (19), although increased tumourigenesis was not observed in other reports (25,99). However, on a mismatch repair-deficient (Mlh1 −/−) background, ATR heterozygozity caused a significant increase in tumourigenesis (100). CHK1 heterozygous (+/−) mice were prone to tumourigenesis on a WNT-1 transgenic background (22) and CHK1 heterozygosity induced in mouse mammary glands using a Cre/loxP system caused induction of mammary tumours in a p53 heterozygous background (101). It seems plausible that replication associated DNA damage due to insufficient CHK1 or ATR levels in S phase caused by hypomorphic mutations in these mice could contribute to promote genomic instability and tumour progression.

On the other hand, WEE1 and CHK1 have also been reported to be overexpressed in human cancers. WEE1 is overexpressed in human glioblastoma and a subset of breast cancers (102,103), and CHK1 mRNA expression was elevated in MYC-amplified neuroblastoma (104). Based on their role in restraining CDK activity, high levels of WEE1 and CHK1 would be expected to suppress rather than to promote cell growth. It may therefore seem like a paradox that high levels of CHK1 or WEE1 are found in human tumours where we rather would expect a selective pressure towards genetic alterations allowing uncontrolled growth. An explanation may be that these tumours contain other genetic alterations that promote increased CDK activity and replication stress, and if the CDK activity was too high, the replication associated damage would reach a level of severity resulting in cell

death. These tumours may thus depend on the high levels of WEE1 or CHK1 to maintain CDK activity at sufficiently low levels for cell survival. Consistent with this hypothesis, inhibition of WEE1 leads to induction of DNA damage and cell death in tumours expressing high WEE1 levels (102). Furthermore, MYC is known to cause replication stress and elevated CHK1 expression was found selectively in MYC-amplified neuroblastoma (104). It is very possible that these cells depend on CHK1 to tolerate the high level of MYC expression.

Can the replicative roles of the checkpoint kinases be exploited for cancer treatment?

Checkpoint kinases guard against cancer-associated replication stress**.** In human tumours, replication stress induced by oncogenes or hypoxia leads to increased activation of ATR/CHK1 in S phase cells (105–107). Inhibitors of checkpoint kinases may therefore lead to selective killing of cancer cells with elevated replication stress (Figure 4). Currently, several inhibitors of CHK1 and WEE1 are in clinical trials (108–110); however this is largely based on their G2 checkpoint abrogation function and consequent induction of mitotic catastrophe. When used in combination with chemotherapeutic agents or radiation, inhibition of CHK1 or WEE1 can cause selective sensitization of p53 negative cells (111–116). It has been proposed that p53-negative cancer cells are particularly sensitive to G2 checkpoint abrogation because they lack the p53-dependent G1 checkpoint and therefore will depend more on the G2 checkpoint for DNA damage repair (117). On the other hand, p53-status does not always predict responses to CHK1 inhibition (118–121). Hence, additional effects of CHK1 inhibition, such as high levels of replication stress due to unscheduled initiation, likely contribute to cause cell death (11,27,104,122,123). Following inhibition of WEE1 or CHK1, DNA breakage in S phase (24,26,27) likely will cause killing of cancerous as well as normal tissue cells. Upon inhibition of these checkpoint kinases, loss of their function in restraining CDK activity might therefore contribute to cause unwanted normal tissue damage. However, the tumours would often contain a higher fraction of cycling cells than the surrounding normal tissue, as well as elevated replication stress due to genetic alterations (105,106) or hypoxia (107). The deleterious S phase effects in response to inhibition of these checkpoint kinases may therefore likely be more severe in the tumour compared to normal tissues, resulting in tumour selectivity. Moreover, treatments with checkpoint kinase inhibitors would benefit from a genetic profiling of the cancer, as this could allow pre-selection of patients with cancers that harbour marked replication stress.

In agreement with such tumour selective effects in S phase following checkpoint kinase inhibition, non-transformed mammary epithelial cells showed less γ-H2AX staining, less replication issues and less apoptosis compared to breast cancer cell lines in response to WEE1 inhibition (124). Inhibition of ATR can also sensitize cancer cells and ATR has also been suggested as a therapeutic target based on its role in restraining replication, although small-molecule ATR-inhibitors have only recently started to become available (75,125). The possibility of gaining tumour selective treatment based on inhibiting checkpoint kinases to induce deleterious DNA damage selectively in S phase tumour cells is an exciting task for future studies.

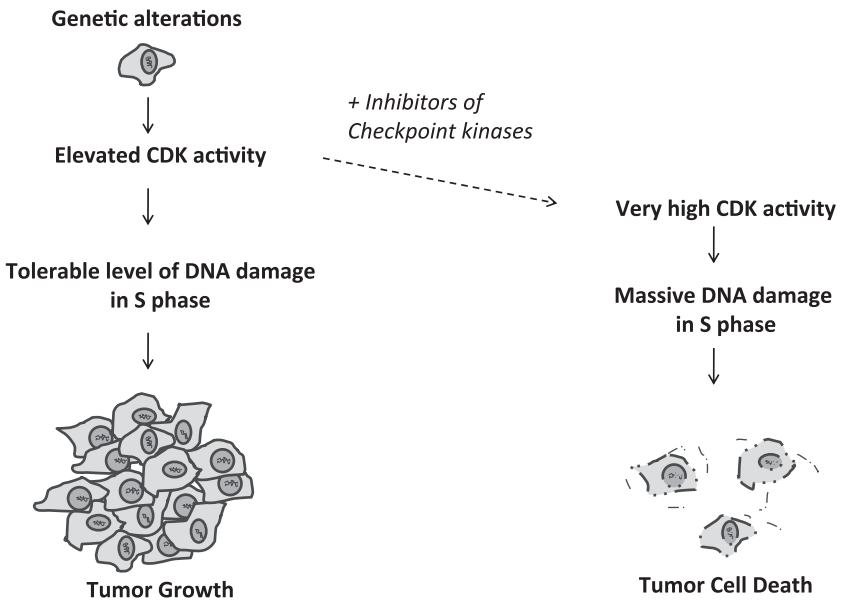

Figure 4. S phase effects during cancer treatment with inhibitors of ATR, CHK1 or WEE1 kinases may promote cancer specific killing. Cancer cells often contain elevated CDK activity due to genetic alterations, which may induce replication failures leading to DNA damage in S phase and subsequent genomic instability and tumour progression. Treatment with inhibitors of ATR, CHK1 or WEE1 further increases CDK activity leading to massive CDK-mediated DNA damage in S phase and subsequent cell death of S phase cancer cells.

CONCLUSION

The DNA replication process is highly regulated with a large number of control mechanisms securing correct timing and quality of the process. Recent work has revealed that the checkpoint kinases ATR, CHK1 and WEE1 are active during normal S phase progression in the absence of exogenous DNA damage. Their activities are critical to maintain genome integrity, and they largely control genome integrity by restraining CDK activity. Deregulated CDK activity will cause unscheduled firing of replication origins in S phase and thereby lead to the induction of DNA breaks in a not yet fully understood mechanism. Such replication-associated DNA lesions may contribute to promote loss of genome integrity and cancer progression following heterozygous mutations or other ways of inactivation of ATR, CHK1 or WEE1 during tumorigenesis. Furthermore, replication-associated DNA damage occurring in response to small-molecule inhibitors of ATR, CHK1 and WEE1 may be exploited to identify new potential strategies for tumour selective treatment.

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