CANCER DIAGNOSIS & PROGNOSIS
3: 639-648 (2023) doi: 10.21873/cdp.10266

Review

The Role of γH2AX in Replication Stress-induced Carcinogenesis: Possible Links and Recent Developments

MICHALIS FRAGKOS, MARIA CHOLEZA and PARASKEVI PAPADOPOULOU

Department of Science and Mathematics, Deree-The American College of Greece, Athens, Greece

Abstract. *Cancer is a condition characterized by genomic instability and gross chromosomal aberrations. The inability of the cell to timely and efficiently complete its replication cycle before entering mitosis is one of the most common causes of DNA damage and carcinogenesis. Phosphorylation of histone 2AX (H2AX) on S139 (γH2AX) is an indispensable step in the response to DNA damage, as it is required for the assembly of repair factors at the sites of damage. γH2AX is also a marker of DNA replication stress, mainly due to fork collapse that often follows prolonged replication stalling or repair of arrested forks, which involves the generation of DNA breaks. Although the role of γH2AX in the repair of DNA breaks has been well defined, the function of γH2AX in replicative stress remains unclear. In this review, we present the recent advances in the field of replication stress, and highlight a novel function for γH2AX that is independent of its role in the response to DNA damage. We discuss studies that support a role for γH2AX early in the response to replicative stress, which does not involve the repair of DNA breaks. We also highlight recent data proposing that γH2AX acts as a chromatin remodeling component, implicated in the efficient resolution of stalled replication forks. Understanding the mechanism by which γH2AX enables cellular recovery after replication stress will allow identification of novel cancer biomarkers, as well as new targets for cancer therapies.*

Correspondence to: Michalis Fragkos, Ph.D., Assistant Professor and Head, Department of Science and Mathematics, Deree-The American College of Greece, Gravias 6, Aghia Paraskevi, Athens, 15342, Greece. Tel: +30 2106009800 ext. 1388, e-mail: mfragkos@acg.edu

Key Words: DNA replication stress, cancer, DNA damage, γH2AX, biomarker, review.

DNA replication is the molecular process that allows the faithful and timely duplication of the cell's genome (1). In contrast to budding yeast, where DNA replication originates at AT-rich sequences, metazoan origins of replication have not yet been fully characterized. Metazoan cells have an enormous number of replication origins that are licensed for replication prior to S-phase. However, only a few of them will be used, allowing the flowless and on-time replication of the genome well before entering the mitotic phase. Initiation of DNA replication occurs in two steps. At first, replication origins are licensed by the assembly of a series of protein factors that form the pre-replication complex (pre-RC). Then, only a few of the licensed origins will be activated, by forming a second protein complex called the pre-initiation complex (pre-IC), which will then allow the synthesis of DNA to proceed (1, 2). Therefore, there is a high degree of flexibility in replication origin activation, allowing cells to perpetually adapt to alterations in their gene expression programs and stage of differentiation.

There is a series of challenges of both intracellular and extracellular origin that may contribute to replication fork slowing, stalling, or even collapsing, causing DNA replication stress. The main endogenous sources of replication stress include conflicts between replication and transcription, as well as the presence of difficult-to-replicate genomic regions. These regions may include G4-rich motifs or repetitive and heterochromatic sequences, such as centromeric, telomeric and ribosomal DNA (rDNA) sequences (3, 4). Replication stress can also be caused by defects in chromatin assembly, activation of oncogenes that may modify the origin usage program of the cell, free radicals such as the reactive oxygen species (ROS) and exogenous factors, like UV-light or genotoxic chemicals. The

cell, however, possesses a well-organized cascade of factors to respond to replicative stress, allowing it to repair any genomic damage that might have been caused before or during replication and, most importantly, resume replication before completing mitosis (2, 3, 5–7). This mechanism is essential for preventing the transformation of healthy cells into cancerous ones.

The response to stalled replication forks includes a significant number of protein factors that may also be used as markers of replicative stress. Histone 2AX (H2AX) is a mammalian variant of histone 2A that, depending on the cell type, accounts for up to 25% of total H2A (8). Following DNA damage, H2AX is phosphorylated on its carboxyterminal end, specifically on serine 139, to generate γH2AX (9). H2AX is phosphorylated by kinases of the phosphoinositide 3-kinase (PI3K) family and, in case of DNA damage caused by replication stress, it is mainly phosphorylated by the ataxia telangiectasia and Rad3-related (ATR) kinase (10, 11). Once γH2AX is formed on chromatin, it binds to mediator of DNA damage checkpoint 1 (Mdc1), which in turn amplifies further the phosphorylation of H2AX at the γ-site, extending the γH2AX signal up to a megabase away from the site of damage, spanning both sides of the damaged site. The assembly of the Mdc1-γH2AX complex is then followed by the attraction of DNA repair factors, the most important being the Mre11-Rad51-Nbs1 (MRN) complex, breast cancer 1 (BRCA1) and p53-binding protein 1 (53BP1) (12-14). γH2AX is considered to give time to the repair factors to function by holding the broken ends of DNA open (15). H2AX-defective mice are viable, but growth retarded and prone to genomic instability and cancer (16). This indicates that γ-H2AX in not essential for cell viability, but is required for the faithful and prompt repair of DNA damage. Taken together, γH2AX has been shown to serve as an indispensable factor for the efficient repair of DNA breaks.

γH2AX phosphorylation is also an indispensable component of the response to replicative stress (11). This may be attributed to the fact that replication stalling is often followed by fork reversal and collapse, leading to the generation of DNA breaks that activate the DNA damage response, causing γH2AX phosphorylation. Although this is a well-established theory to explain the replication stress-induced γH2AX, there have been older studies that elaborated the presence of this marker in the absence of DNA breaks (17, 18). In this review we present all the recent data that highlight the importance of γH2AX in the response to replication stress. We also examined whether γH2AX phosphorylation is an early step in the response to replication stress, prior to fork collapse and the formation of DNA breaks, and whether it can serve as a reliable molecular marker of cancer. Our review sheds light on a new function of γH2AX that is independent of its well-established role in the repair of double-stranded DNA breaks.

Recent Developments in Replication Stress

Replication stalling produces long stretches of single-stranded DNA (ssDNA) that attract the ssDNA binding replication protein A (RPA) (4). RPA-bound ssDNA acts as a signaling platform to recruit the ataxia telangiectasia-mutated and Rad-3-related interacting protein (ATRIP) and ATR. Activation of ATR leads to a series of phosphorylation events on many proteins, including H2AX at Ser139, RPA and Checkpoint 1 (Chk1) kinase (3, 4). The ATR-Chk1 pathway is the main pathway activated by stalled replication forks, preventing late origin firing and leading to a timely cell-cycle arrest in S-phase. This allows the cell to unblock the stalled replication forks, resolve DNA replication and repair DNA damage before it enters the mitotic phase. In case this is not feasible, the cell will enter mitosis with DNA breaks and incompletely replicated DNA, causing a defective mitotic cycle characterized by chromosome breaks, bridges and micronuclei (3, 19). Such defects result in significant genomic instability, which constitutes the driving force of cancer (20).

Sources of replication stress. Replication stress is mainly caused by intracellular factors, such as replication-transcription conflicts and DNA synthesis at difficult-to replicate loci, inducing RNA:DNA hybrids called R-loops, secondary DNA structures and stalled replication forks (4, 21, 22). This often leads to incomplete firing of replication origins or, alternatively, to the simultaneous activation of an excessive number of origins, which may lead to exhaustion of replication factors (3). In fact, depletion of proteins involved in the process of replication is a frequent cause of replication stress. The most common factor that is often exhausted in case of massive origin activation is RPA, mainly due to its role in protecting ssDNA by binding to it (23). Moreover, excessive origin firing has been recently shown to be associated with increased mitotic spindle growth, leading to improper chromosome segregation and significant chromosomal instability (24).

A recent study by the group of Jiri Bartek revealed a new source of replication stress (25). By using inhibitors of poly(ADP-ribose) polymerase (PARP), a chromatin-associated enzyme that modifies nuclear proteins involved in DNA repair, Maya-Mendoza *et al*. managed to speed up the process of DNA replication and showed that accelerated replication may result in DNA damage instead of causing replication pausing. This is an important finding, as it reveals that the cell is capable of controlling the speed of DNA replication, ensuring the faithful and prompt duplication of its genome. Moreover, high-speed replication can be the driving force of replication factor exhaustion that often causes replicative stress.

Replication stalling can also be caused by topoisomerase inefficiency that consequently produces a topological stress.

Table I. *List of novel factors involved in the response to DNA replication stress.*

Factor	Name	Function	Reference
ZFP161	Zinc finger protein 161	Transcription regulator	32
SPRTN	SPR T-line N-terminal domain	Metalloendopeptidase	33
NSMF	N-methyl D-aspartate receptor synaptonuclear signaling and neuronal migration factor	Neuron development factor	35
RNR	Ribonucleotide reductase	Nucleotide synthesis enzyme	37
USP13	Ubiquitin-specific protease 13	Deubiquitinating enzyme	38
Polι	DNA polymerase iota	DNA synthesis enzyme	39
CRMP2	Collapsin response mediator protein-2	Neuron development factor	40
BTR complex	BLM-TOP3A-RMI1-RMI2 complex	Homologous recombination factor	41
PP2A	Protein phosphatase 2A	Protein phosphatase	42
SETD2	Suppressor of variegation, enhancer of zeste and Trithorax domain containing 2	Histone methyltransferase	34
MCM8/9	Mini-chromosome maintenance 8/9	Helicase	36

A recent study in S. cerevisiae showed that cohesin, a protein that is involved in sister chromatid cohesion, may induce topological stress and therefore impede the repair of the damage induced by replication stress (26). Another important work using the budding yeast as a model was the study by Salim *et al*. This study showed that replication stress may cause S. cerevisiae to progressively shorten its rDNA array (27). rDNA is a long repetitive genomic sequence composed of a large number of tandem repeats that is overall prone to replicative stress. The study revealed that contraction of the rDNA array is a physiological response to replication stress, as it liberates replication stress factors that may then be used in other parts of the genome. Finally, another recent study in yeast, where a site-specific DNA replication barrier of bacterial origin was used, revealed that unresolved replication stress may lead to DNA deletions, as well as duplications (28).

An important advance in the field of replication stress was the finding that heads-on collisions between transcription and replication are regulated by Topoisomerase I (Top1), which acts to prevent genomic instability (29). The group of Philippe Pasero showed that Top1 is necessary for replication fork pausing at the transcription terminators of actively transcribing genes. This mechanism protects from transcription-replication collisions and allows efficient resolution of DNA replication, therefore preventing fork collapse, DNA damage and chromosome breaks. In addition, reactive oxygen species (ROS) were recently shown to induce replicative stress by enhancing replication-transcription conflicts and R-loop formation (30). Moreover, the group of Valeria Naim identified Senataxin (SETX), an RNA:DNA helicase involved in the resolution of R-loops, as a factor protecting cells from transcription-associated replication stress (31). Collectively, the aforementioned studies confirmed the existing causes of replicative stress and enabled us to comprehend their underlying mechanisms.

Novel factors involved in the resolution of replication stress. Recent developments in the field of DNA replication stress include the discovery of a series of novel DNA repair factors (summarized in Table I). The Zinc-finger protein ZFP161 is a transcription regulator found to mediate the interaction between RPA and ATR/ATRIP in response to replicative stress (32). In addition to that, the group of Kristijan Ramadan showed that the metalloendopeptidase SPRTN (SPR T-line N-terminal domain) is involved in the resolution of stalled forks by interacting with Chk1 and activating the ATR-Chk1 pathway (33). Moreover, Zhu *et al*. demonstrated that RPA binding to chromatin is mediated by the histone methyltransferase SETD2 (34). Histone methylation was shown to be essential for the activation of the ATR signaling pathway. Another factor that was recently reported to be involved in replication fork restart is N-methyl D-aspartate receptor synaptonuclear signaling and neuronal migration factor (NSMF). This protein, involved in neuronal plasticity and development, was shown to have a role in the ATR/ATRIP pathway by allowing the interaction between RPA and ATRIP on single-stranded DNA produced by stalled forks (35). Replication fork integrity was also shown to be achieved by the MCM8/9 complex (36). MCM8/9, which acts as a helicase for homologous recombination, plays an unexpected role in protecting stalled replication forks, maintaining genomic stability under conditions of replicative stress.

Ribonucleotide reductase (RNR) is another factor recently implicated in the response to DNA replication stalling (37). RNR is an enzyme involved in nucleotide synthesis and is phosphorylated on S559. Defective RNR phosphorylation was shown to lead to replication stress and subsequent genomic instability. Another enzyme that was recently shown to play a role in the resolution of arrested forks is USP13. This is a deubiquitinating enzyme shown to interact with and stabilize topoisomerase IIβ-binding protein 1 (TopBP1), a protein with

a well-known role in the response to replicative stress (38). Amongst the novel components in this type of response is also DNA polymerase iota (Pol ι). In a study to reveal the factors that compensate for the absence of the Fanconi anemia (FA) pathway, Wang *et al*. showed that Pol ι is an indispensable factor for the resolution of stalled replication forks in the absence of a functional FA pathway (39).

A recent study by the Loizou lab revealed a role for a new factor, called Collapsin response mediator protein-2 (CRMP2), in the cellular response to replication stress (40). By using a phosphoproteomics approach based on mass spectrometry, they showed that aphidicolin-induced replication stress leads to S522 phosphorylation of CRMP2 in an ATM/ATMIN dependent manner. Given that CRMP2 is massively phosphorylated at S522 in Alzheimer's patients, this indicates that the cellular damage observed in the brain of Alzheimer's patients may be, at least partially, attributed to replication stress. Amongst other factors that have been recently studied is the BTR complex, composed of the Bloom syndrome helicase (BLM), topoisomerase IIIα and RecQ mediated instability proteins 1 and 2 (41). Although this complex has an essential role in homologous recombination, the study by Shorrocks *et al*. showed that the BTR complex is indispensable for recruiting BLM at sites of fork restart, via the single-stranded DNA binding protein RPA. Finally, a study in budding yeast revealed that protein phosphatase 2A (PP2A), a protein that contributes to mitotic arrest by dephosphorylating key regulators of the cell cycle, is a factor that prevents anaphase entry prior to resolution of arrested forks, rendering PP2A an indispensable factor for preventing genomic instability (42).

Non-coding RNAs and replication stress. Non-coding RNAs have been shown to have a role in the response to DNA damage, as they are involved in the assembly of DNA repair foci (43). Moreover, the miRNA pathway was shown to be indispensable for the response to replication stress, as depletion of Dicer led to inhibition of the S-phase checkpoint, allowing the cells to enter mitosis with under-replicated DNA (44). A study by the group of Nick Proudfoot showed that inactivation of the RNA polymerase II-associated histone chaperone SPT6 leads to H3K36me3 of long non-coding RNA (lncRNA) genes, leading to the production of very long lncRNA transcripts that hybridize with single-stranded DNA, causing the formation of R-loops and subsequent replication stress (45). A similar study showed that ncRNA transcription from repetitive centromeric sequences may cause conflicts with DNA replication, in the absence of the centromeric H3 variant CENP-A (46). On the other hand, another study revealed a novel lncRNA called Discn that has a determining role in the resolution of stalled replication forks (47). Discn functions by binding to nucleolin, an RPA-interacting protein, preserving the pool of RPA for the response to replication stress. Taken together, these data show that ncRNAs have a role in protecting the cell from replicative stress, however, in some cases the expression of lncRNAs may provoke replication stalling and genomic instability.

The Role of γH2AX in Replication Stress: Recent Advances

γH2AX is a widely established chromatin signal of DNA damage. Given that DNA replication stress, if unresolved, results in fork collapse and DNA breaks, γH2AX may serve as a marker of replicative stress (11). An appropriate cellular model for studying replication-related γH2AX phosphorylation is the use of stem cells, which often show constitutive replication stress. For example, old hematopoietic stem cells (HSCs) characterized by DNA replication defects, show intense γH2AX staining in the nucleolus that can be attributed to inefficient H2AX dephosphorylation by the corresponding phosphatase, highlighting the persistent replication stress observed in rDNA genes (48). A study by Ahuja *et al*. showed that cultured embryonic stem cells are positive for several markers of replication stress, including γH2AX, in the absence of any treatment (49). In this study, constitutive replication stress was attributed to a rapid G1-to-S transition. Another recent work in which γH2AX served as a marker of replication stress is the study by Vallabhaneni *et al*., where induced pluripotent stem cells (iPSCs) that perform rapid replication cycles were used (50). The iPSCs showed massive amounts of phosphorylated H2AX at S139, a result of excessive levels of double-stranded DNA breaks as a consequence of high-speed replication. This study is in line with the work by Maya-Mendoza *et al*. presented above, which showed that accelerated replication contributes to replication stress.

A thorough genome-wide analysis of the sites that develop the γH2AX signal following replication stress was performed by the group of Weihang Chai. In this study, replication stress was induced by chemical agents (aphidicolin, hydroxyurea and methyl methanesulfonate) and γH2AX sites were mapped using CHIP sequencing (CHIP-seq) (51). The study verified that replication stress is induced at difficult-to-replicate loci, such as large genes and common fragile sites. In addition, it revealed replication stress hotspots in short interspersed nuclear elements (SINEs), but not in long interspersed nuclear elements (LINEs), long terminal repeats (LTRs) or other DNA transposable elements. Moreover, replication stress-induced γH2AX hotspots were identified in chromatin with compact characteristics, as the γH2AX hotspots coincided with heterochromatic epigenetic markers, such as H3K27me3 and H3K9me3. This study provides a global view of the genomic regions that are prone to replication stalling, verifying expected hotspots and revealing new ones.

A similar work also based on γH2AX CHIP-Seq led to the identification of the transcription regulator bromodomain-containing protein 4 (BRD4) as a new contributor in the resolution of transcription-replication collisions, R-loops and therefore DNA replication stress (52). This study made use of the γH2AX marker to identify sites of R-loop-induced replication stress in the absence of BRD4. Taken together, the studies based on γH2AX CHIP-Seq may serve as a guide for identifying novel sites of genomic instability induced by replicative stress.

γH2AX phosphorylation independent of replication stress-induced DNA breaks. It is widely accepted that phosphorylation of H2AX is a consequence of double-stranded DNA break formation caused by replication fork reversal, repair, or collapse. However, there is accumulating evidence that H2AX may be phosphorylated prior or independently of DNA break formation (17, 53). Katsube *et al*. performed a study to investigate whether replication stress-induced γH2AX phosphorylation is a result of double-stranded DNA break formation (54). The group investigated the DNA damage response to ionizing radiation, which induces double-stranded DNA breaks, and to hydrogen peroxide that causes oxidative stress and subsequently replicative stress. They reported that oxidative stress-induced γH2AX was observed in two phases, with wide nuclear foci appearing soon after treating the cells with hydrogen peroxide, some of which reappeared 24 h after the treatment. The γ-phosphorylation of H2AX was dependent on ATR, suggesting that this response was induced by stalled replication forks. Most of the oxidative stress-induced γH2AX foci did not colocalize with phospho-ATM or 53BP1, supporting the idea that this type of H2AX phosphorylation is not induced by the formation of double-stranded DNA breaks. This study therefore supports the idea that ATR-dependent phosphorylation of H2AX at S139 takes place prior and independently of the formation of double-stranded DNA breaks.

Another study that identified a new role for γH2AX in DNA replication stress is the work by the group of Barry Sleckman. This group found that γH2AX prevents fork reversal at sites of stalled replication forks and inhibits the activation of a DNA damage response (55). They also showed that H2AX functions along with the XRCC4-like factor (XLF), an unexpected finding since XLF is a critical factor in non-homologous end joining and the repair of double-stranded DNA breaks. Moreover, H2AX and XLF double mutant cells exhibited an absolute requirement of ATR for the resolution of replication stress and survival. Taken together, this study presents a role for γH2AX well before fork collapse and formation of DNA breaks, supporting the idea that γ-phosphorylation of H2AX in replication stress is required before the DNA damage-induced response.

Another study that clearly addressed the question of whether γ-phosphorylation of H2AX marks stalled forks independently of its role in the resolution of double-strand DNA breaks, was the work by Moeglin *et al*. This study demonstrated that excessive replication stress induces pan-nuclear phosphorylation of H2AX at S139, mediated by DNA-dependent protein kinase (DNA-PK) (56). This work is in line with previous studies showing that kinase overactivation may result in a pan-nuclear pattern of γH2AX phosphorylation (17, 53). In the study by Moeglin *et al*., the γH2AX signal coincided with the hyperphosphorylation of the single-stranded binding protein RPA. It is therefore tempting to speculate that γ-phosphorylation of H2AX in stalled replication forks derives from kinase overactivation that does not only phosphorylate DNA-bound RPA but also H2AX, located at the stalled forks. This is another study providing evidence that γH2AX is not only a marker of double-stranded DNA breaks, but can also serve as a signal of replication stress independently of DNA damage.

The effect of γH2AX on chromatin and nuclear restructuring. γ-Phosphorylation of H2AX is a nuclear response required for the effective repair of DNA damage. Moreover, chromatin needs to become reorganized in order to respond to problems related to DNA replication (57). However, the way by which γH2AX functions in the resolution of stalled replication forks is still unclear. Singh *et al*. revealed that lamins A and C, two structural proteins that determine the shape of the nucleus, are required for the efficient response to replication stress (58). Using lamin A/C-deficient cells, the study illustrated that a nuclear lamin network serves as a platform for resolving stalled replication forks. In particular, by inducing replication stress with hydroxyurea, in the absence of lamin A/C, they identified a delay in γH2AX foci clearance and defective recruitment of repair factors onto replication foci. They also demonstrated increased genomic instability following replication stress in the absence of lamin proteins, by identifying a significant number of triradial chromosomes. Given that in the absence of Lamin A/C persistence of γH2AX on chromatin prevents the binding of DNA repair factors, this work also supports the idea that γ-phosphorylation of H2AX following replication stalling is induced prior to DNA damage and is not only involved in the repair of DNA breaks. Collectively, these results show that the shaping of the nucleus is an important requirement for the efficient resolution of stalled replication forks and that this process involves components such as Lamins and γH2AX.

Another important finding regarding the role of γ-phosphorylation of H2AX on replicative stress comes from the study by Kim *et al*., where they demonstrate that γH2AX phosphorylation is followed by the deposition of the macroH2A1.2 histone variant at sites of stalled replication forks (59). MacroH2A1.2 binding to chromatin depends on the Facilitates Chromatin Transcription (FACT) complex and results in attracting the homologous recombination factor BRCA1. The accumulation of macroH2A1.2 on chromatin

following replication stress spans several hundred kilobases and therefore acts, similar to γH2AX, as a platform for attracting DNA repair factors. Overall, this study reveals a determining role for macroH2A1.2 in the resolution of stalled replication forks and further supports a role for γH2AX in the response to replication stress, independent of its role in the resolution of DNA breaks.

Another study that examined the role of H2AX in chromatin restructuring prior to the response to replication stress, was the work by Seo *et al*. (60). H2AX and its consequent γ-phosphorylation were found enriched in subtelomeric and early replicating fragile sites that are characterized by high transcriptional activity and therefore open chromatin environment. However, and in contrast to the genome-wide study by Lyu *et al*. (51), heterochromatic regions that contain common fragile sites were not found to be enriched in H2AX (60). This study also revealed the colocalization of H2AX with the chromatin remodeling factor INO80 under conditions of replication stress, suggesting a potential role for INO80 in the removal of γH2AX and its replacement by non-phosphorylated H2AX. Taken together, this study supports an H2AX-mediated mechanism of chromatin restructuring to allow for the resolution of stalled replication forks.

Lastly, γH2AX is also implicated in chromatin modification for transcription initiation. Dobersch *et al*. have shown that H2AX phosphorylation at S139 is a prerequisite for chromatin opening to initiate the process of transcription (61). The opening of chromatin prior to transcription required the demethylation of DNA, mediated by a DNA repair apparatus that includes γH2AX. This may provide an explanation for the necessity of γH2AX in transcriptional activation. Given the fact that replication stress can be caused by transcription-replication collisions, it would be interesting to investigate whether such a mechanism would also apply in transcription re-initiation after replication stress-induced transcription-replication conflicts.

Replication stress-induced γH2AX and cancer. Cancer cells are characterized by persistent replication stress, indicating that the proteins involved in the resolution of stalled replication forks are likely to be defective. A significant milestone in understanding the molecular basis of cancer was the finding that oncogene activation leads to replication stress, subsequently inducing DNA damage and carcinogenesis (62). A follow-up work showed that activation of oncogenes results in the firing of intragenic replication origins that are present in highly transcribed genomic regions. This results in transcription-replication collisions, DNA breaks and genomic instability, providing a mechanism that can explain how oncogene-induced replication stress may eventually lead to cancer (63). In addition to this work, a recent study demonstrated that the RAD18/Polκ (DNA polymerase kappa) pathway is involved in preventing the replicative stress induced by the CDK2 oncogene (64). If the response to oncogene-induced replication stress is defective, cells may adapt a stem-like cancerous state, losing both their proliferation control and their differentiation status (65). Moreover, oncogene-induced replication stress was shown to contribute to mitotic non-random chromosomal segregation, a process that sustains the genomic integrity of cancer cells and may provide an explanation for the tolerance of replicative stress in cancer cells (66).

Guerrero Llobet *et al*. revealed a new group of oncogenes that are activated in cancer cells exhibiting oncogene-induced replication stress. Amongst these oncogenes is an acetyltransferase named NAT10, whose expression correlates with replication stress markers, such as γH2AX and phospho-RPA (67). NAT10 could have a role in chromatin restructuring, allowing cancer cells to cope with constitutive replication stress. In addition to these studies, there was a recent interesting finding about the role of the sonic hedgehog (SHH) pathway in cancer (68). SHH is an important morphogen that regulates metazoan embryogenesis and development, whereas its uncontrolled expression may lead to carcinogenesis. The study by Tamayo-Orrego *et al*. revealed that Shh-induced carcinogenesis is caused by DNA helicase overloading and increased replication origin firing. Taken together, the above studies aim at providing a mechanistic approach to the oncogene-induced replicative stress, which will allow the identification of novel approaches to treat cancer.

γH2AX as a Marker of Replication Stress and Cancer

DNA replication stress is a common characteristic of precancerous lesions as well as in a variety of different solid cancers (69, 70). Early prognosis of cancer is very important in preventing metastasis and progression to lethal stages of cancer. For this reason, the identification of reliable biomarkers in early cancer samples has been a field of intense research during the last few years. These biomarkers must be sensitive, specific, robust, but also cost-effective and rapidly identifiable, so as to be used not only in the diagnosis, but also the prognosis of cancer. It has been therefore tempting to investigate whether any of the factors involved in the response to replication stress can potentially be used as biomarkers of cancer. To this end, the group of Ying Liu did a thorough work using various replication stress factors and, by performing immunohistochemical studies, investigated the presence of these factors in colon, lung, breast and stomach cancer samples (70). This study verified the presence of markers such as cyclin E and DNA Polymerase D3 in solid cancers, as most of the samples were positive for these markers. However, protein markers that are known to be involved in the response to replication stress, such as γH2AX and FANCD2, were found only in a few of

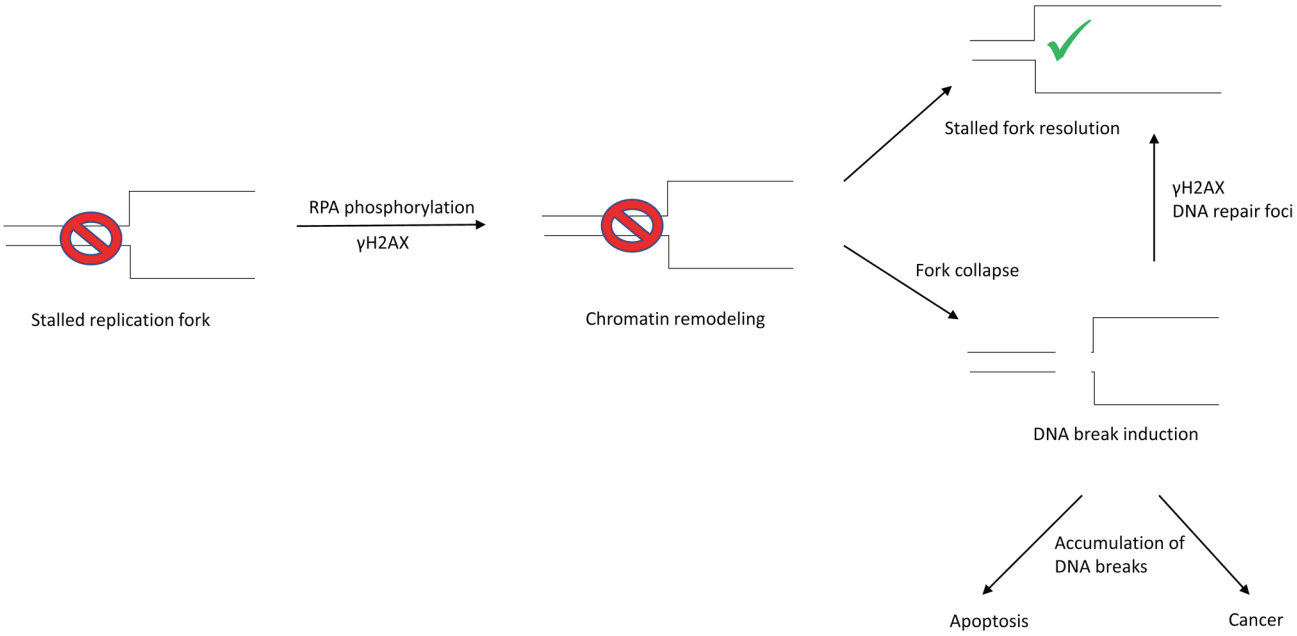

Figure 1. *Summary of the role of γH2AX in the response to replicative stress. H2AX is phosphorylated soon after the phosphorylation and binding of RPA on single-stranded DNA. γH2AX allows the fast resolution of stalled forks via chromatin restructuring. If this is not achieved, or in the case of fork collapse, DNA breaks are generated and H2AX is phosphorylated again. In this case, γH2AX mediates the assembly of repair factors on chromatin, so as to resolve the arrested forks and allow the completion of replication. If this is not possible, the cell will accumulate DNA breaks that will eventually trigger apoptosis or, in the absence of a functional apoptotic pathway, carcinogenesis.*

the samples. This can be attributed to the fact that replication stress is mainly seen in early-stage cancer samples, whereas this study used mostly late-stage solid tumor samples. Moreover, there was no correlation between the γH2AX and the FANCD2 markers, suggesting that none of the two could serve as a reliable biomarker of solid tumor samples. A similar study assessed three protein factors, phospho-RPA32, γH2AX and 53BP1, as biomarkers of oncogene-induced and ROS-induced replicative stress (71). Even though pRPA32 was found to be the most consistent marker, γH2AX was also shown to serve as a reliable biomarker of replication stress. Taken together, these studies show that even though γH2AX is a well-established marker for assessing the genotoxicity of carcinogenic agents, it may not be the most reliable and robust biomarker for identifying solid cancer samples (72).

Conclusion

The molecular mechanism of carcinogenesis has been an intense field of research during the last few decades. A defective replication process and the inability to resolve arrested replication forks may push the cell with incompletely replicated DNA into mitosis, leading to chromosomal breaks and genomic instability. An important chromatin mark that characterizes replication stress is the phosphorylation of H2AX at S139. This mark comes as a result of the formation of DNA breaks, following fork reversal or collapse. However, there has been a series of recent studies supporting the idea that γH2AX may have a function early in replication stress, independently of its role in mediating the repair of DNA breaks. Moreover, a series of studies reveal a role of γH2AX in chromatin restructuring, proposing an alternative mechanism for the function of γH2AX in replicative stress. As illustrated in Figure 1, we propose that H2AX is phosphorylated at Ser139 soon after replication stalling to initiate a series of chromatin modifications that restructure the nucleus, allowing the effective re-initiation of replication. If this process fails, γH2AX will reappear after fork collapse, mediating the repair of DNA damage (Figure 1). Future studies should be directed towards understanding this novel function of γH2AX, identifying the factors that interact with γH2AX and investigating the nuclear restructuring required for the effective resolution of stalled replication forks. Intensive research on the role of γH2AX in replication stress will allow us identify novel factors that can be used either as biomarkers for the prognosis and diagnosis of cancer or directly as a means to target and eliminate cancer cells.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

MF conceived, designed, did the literature review, and wrote the article. PP prepared the references for the paper. MF, MC, and PP evaluated the selected literature review articles and revised critically the paper.

Funding

Funding is provided by the American College of Greece.

References

1 Fragkos M, Ganier O, Coulombe P, Méchali M: DNA replication origin activation in space and time. Nat Rev Mol Cell Biol 16(6): 360-374, 2015. DOI: 10.1038/nrm4002
2 Bell SP, Dutta A: DNA replication in eukaryotic cells. Annu Rev Biochem 71(1): 333-374, 2002. DOI: 10.1146/annurev.biochem.71.110601.135425
3 Fragkos M, Naim V: Rescue from replication stress during mitosis. Cell Cycle 16(7): 613-633, 2017. DOI: 10.1080/15384101.2017.1288322
4 Zeman MK, Cimprich KA: Causes and consequences of replication stress. Nat Cell Biol 16(1): 2-9, 2014. DOI: 10.1038/ncb2897
5 Minocherhomji S, Ying S, Bjerregaard VA, Bursomanno S, Aleliunaite A, Wu W, Mankouri HW, Shen H, Liu Y, Hickson ID: Replication stress activates DNA repair synthesis in mitosis. Nature 528(7581): 286-290, 2015. DOI: 10.1038/nature16139
6 Berti M, Vindigni A: Replication stress: getting back on track. Nat Struct Mol Biol 23(2): 103-109, 2016. DOI: 10.1038/nsmb.3163
7 Branzei D, Foiani M: Maintaining genome stability at the replication fork. Nat Rev Mol Cell Biol 11(3): 208-219, 2010. DOI: 10.1038/nrm2852
8 Kinner A, Wu W, Staudt C, Iliakis G: Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. Nucleic Acids Res 36(17): 5678-5694, 2008. DOI: 10.1093/nar/gkn550
9 Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM: DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273(10): 5858-5868, 1998. DOI: 10.1074/jbc.273.10.5858
10 Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A: H2AX: the histone guardian of the genome. DNA Repair 3(8-9): 959-967, 2004. DOI: 10.1016/j.dnarep.2004.03.024
11 Georgoulis A, Vorgias CE, Chrousos GP, Rogakou EP: Genome instability and γH2AX. Int J Mol Sci 18(9): 1979, 2017. DOI: 10.3390/ijms18091979
12 Palla VV, Karaolanis G, Katafigiotis I, Anastasiou I, Patapis P, Dimitroulis D, Perrea D: gamma-H2AX: Can it be established as a classical cancer prognostic factor? Tumour Biol 39(3): 101042831769593, 2017. DOI: 10.1177/1010428317695931
13 Fernandez-Capetillo O, Celeste A, Nussenzweig A: Focusing on Foci: H2AX and the recruitment of DNA-damage response factors. Cell Cycle 2(5): 425-426, 2003. DOI: 10.4161/cc.2.5.509
14 Lukas C, Savic V, Bekker-Jensen S, Doil C, Neumann B, Sølvhøj Pedersen R, Grøfte M, Chan KL, Hickson ID, Bartek J, Lukas J: 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. Nat Cell Biol 13(3): 243-253, 2011. DOI: 10.1038/ncb2201
15 Bassing CH, Alt FW: The cellular response to general and programmed DNA double strand breaks. DNA Repair (Amst) 3(8-9): 781-796, 2004. DOI: 10.1016/j.dnarep.2004.06.001
16 Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Olaru A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, Nussenzweig A: Genomic instability in mice lacking histone H2AX. Science 296(5569): 922-927, 2002. DOI: 10.1126/science.1069398
17 Fragkos M, Jurvansuu J, Beard P: H2AX is required for cell cycle arrest via the p53/p21 pathway. Mol Cell Biol 29(10): 2828-2840, 2009. DOI: 10.1128/MCB.01830-08
18 Pospelova TV, Demidenko ZN, Bukreeva EI, Pospelov VA, Gudkov AV, Blagosklonny MV: Pseudo-DNA damage response in senescent cells. Cell Cycle 8(24): 4112-4118, 2009. DOI: 10.4161/cc.8.24.10215
19 Chan KL, Palmai-Pallag T, Ying S, Hickson ID: Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. Nat Cell Biol 11(6): 753-760, 2009. DOI: 10.1038/ncb1882
20 Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller MC, Shaikh N, Domingo E, Kanu N, Dewhurst SM, Gronroos E, Chew SK, Rowan AJ, Schenk A, Sheffer M, Howell M, Kschischo M, Behrens A, Helleday T, Bartek J, Tomlinson IP, Swanton C: Replication stress links structural and numerical cancer chromosomal instability. Nature 494(7438): 492-496, 2013. DOI: 10.1038/nature11935
21 Saxena S, Zou L: Hallmarks of DNA replication stress. Mol Cell 82(12): 2298-2314, 2022. DOI: 10.1016/j.molcel.2022.05.004
22 Santos-Pereira JM, Aguilera A: R loops: new modulators of genome dynamics and function. Nat Rev Genet 16(10): 583-597, 2015. DOI: 10.1038/nrg3961
23 Toledo L, Neelsen KJ, Lukas J: Replication catastrophe: When a checkpoint fails because of exhaustion. Mol Cell 66(6): 735-749, 2017. DOI: 10.1016/j.molcel.2017.05.001
24 Böhly N, Schmidt A, Zhang X, Slusarenko BO, Hennecke M, Kschischo M, Bastians H: Increased replication origin firing links replication stress to whole chromosomal instability in human cancer. Cell Rep 41(11): 111836, 2022. DOI: 10.1016/j.celrep.2022.111836
25 Maya-Mendoza A, Moudry P, Merchut-Maya JM, Lee M, Strauss R, Bartek J: High speed of fork progression induces DNA replication stress and genomic instability. Nature 559(7713): 279-284, 2018. DOI: 10.1038/s41586-018-0261-5
26 Minchell NE, Keszthelyi A, Baxter J: Cohesin causes replicative DNA damage by trapping DNA topological stress. Mol Cell 78(4): 739-751.e8, 2020. DOI: 10.1016/j.molcel.2020.03.013
27 Salim D, Bradford WD, Freeland A, Cady G, Wang J, Pruitt SC, Gerton JL: DNA replication stress restricts ribosomal DNA copy number. PLoS Genet 13(9): e1007006, 2017. DOI: 10.1371/journal.pgen.1007006

28 Larsen NB, Liberti SE, Vogel I, Jørgensen SW, Hickson ID, Mankouri HW: Stalled replication forks generate a distinct mutational signature in yeast. Proc Natl Acad Sci USA 114(36): 9665-9670, 2017. DOI: 10.1073/pnas.1706640114

29 Promonet A, Padioleau I, Liu Y, Sanz L, Biernacka A, Schmitz AL, Skrzypczak M, Sarrazin A, Mettling C, Rowicka M, Ginalski K, Chedin F, Chen CL, Lin YL, Pasero P: Topoisomerase 1 prevents replication stress at R-loop-enriched transcription termination sites. Nat Commun 11(1): 3940, 2020. DOI: 10.1038/s41467-020-17858-2

30 Andrs M, Stoy H, Boleslavska B, Chappidi N, Kanagaraj R, Nascakova Z, Menon S, Rao S, Oravetzova A, Dobrovolna J, Surendranath K, Lopes M, Janscak P: Excessive reactive oxygen species induce transcription-dependent replication stress. Nat Commun 14(1): 1791, 2023. DOI: 10.1038/s41467-023-37341-y

31 Said M, Barra V, Balzano E, Talhaoui I, Pelliccia F, Giunta S, Naim V: FANCD2 promotes mitotic rescue from transcription-mediated replication stress in SETX-deficient cancer cells. Commun Biol 5(1): 1395, 2022. DOI: 10.1038/s42003-022-04360-2

32 Kim W, Zhao F, Wu R, Qin S, Nowsheen S, Huang J, Zhou Q, Chen Y, Deng M, Guo G, Luo K, Lou Z, Yuan J: ZFP161 regulates replication fork stability and maintenance of genomic stability by recruiting the ATR/ATRIP complex. Nat Commun 10(1): 5304, 2019. DOI: 10.1038/s41467-019-13321-z

33 Halder S, Torrecilla I, Burkhalter MD, Popović M, Fielden J, Vaz B, Oehler J, Pilger D, Lessel D, Wiseman K, Singh AN, Vendrell I, Fischer R, Philipp M, Ramadan K: SPRTN protease and checkpoint kinase 1 cross-activation loop safeguards DNA replication. Nat Commun 10(1): 3142, 2019. DOI: 10.1038/s41467-019-11095-y

34 Zhu Q, Yang Q, Lu X, Wang H, Tong L, Li Z, Liu G, Bao Y, Xu X, Gu L, Yuan J, Liu X, Zhu WG: SETD2-mediated H3K14 trimethylation promotes ATR activation and stalled replication fork restart in response to DNA replication stress. Proc Natl Acad Sci USA 118(23): 2021. DOI: 10.1073/pnas.2011278118

35 Ju MK, Shin KJ, Lee JR, Khim KW, A Lee E, Ra JS, Kim BG, Jo HS, Yoon JH, Kim TM, Myung K, Choi JH, Kim H, Chae YC: NSMF promotes the replication stress-induced DNA damage response for genome maintenance. Nucleic Acids Res 49(10): 5605-5622, 2021. DOI: 10.1093/nar/gkab311

36 Griffin WC, McKinzey DR, Klinzing KN, Baratam R, Eliyapura A, Trakselis MA: A multi-functional role for the MCM8/9 helicase complex in maintaining fork integrity during replication stress. Nat Commun 13(1): 5090, 2022. DOI: 10.1038/s41467-022-32583-8

37 Shu Z, Li Z, Huang H, Chen Y, Fan J, Yu L, Wu Z, Tian L, Qi Q, Peng S, Wei C, Xie Z, Li X, Feng Q, Sheng H, Li G, Wei D, Shan C, Chen G: Cell-cycle-dependent phosphorylation of RRM1 ensures efficient DNA replication and regulates cancer vulnerability to ATR inhibition. Oncogene 39(35): 5721-5733, 2020. DOI: 10.1038/s41388-020-01403-y

38 Kim W, Zhao F, Gao H, Qin S, Hou J, Deng M, Kloeber JA, Huang J, Zhou Q, Guo G, Gao M, Zeng X, Zhu S, Tu X, Wu Z, Zhang Y, Yin P, Kaufmann SH, Luo K, Lou Z: USP13 regulates the replication stress response by deubiquitinating TopBP1. DNA Repair (Amst) 100: 103063, 2021. DOI: 10.1016/j.dnarep.2021.103063

39 Wang R, Lenoir WF, Wang C, Su D, McLaughlin M, Hu Q, Shen X, Tian Y, Klages-Mundt N, Lynn E, Wood RD, Chen J, Hart T, Li L: DNA polymerase ι compensates for Fanconi anemia pathway deficiency by countering DNA replication stress. Proc Natl Acad Sci U S A 117(52): 33436-33445, 2020. DOI: 10.1073/pnas.2008821117

40 Mazouzi A, Stukalov A, Müller AC, Chen D, Wiedner M, Prochazkova J, Chiang S-C, Schuster M, Breitwieser FP, Pichlmair A, El-Khamisy SF, Bock C, Kralovics R, Colinge J, Bennett KL, Loizou JI: A comprehensive analysis of the dynamic response to aphidicolin-mediated replication stress uncovers targets for ATM and ATMIN. Cell Rep 15(4): 893-908, 2016. DOI: 10.1016/j.celrep.2016.03.077

41 Shorrocks AK, Jones SE, Tsukada K, Morrow CA, Belblidia Z, Shen J, Vendrell I, Fischer R, Kessler BM, Blackford AN: The Bloom syndrome complex senses RPA-coated single-stranded DNA to restart stalled replication forks. Nat Commun 12(1): 585, 2021. DOI: 10.1038/s41467-020-20818-5

42 Haluska C, Jin F, Wang Y: Protein phosphatase 2A (PP2A) promotes anaphase entry after DNA replication stress in budding yeast. Mol Biol Cell 32(22): ar36, 2021. DOI: 10.1091/mbc.E21-04-0222

43 Francia S, Michelini F, Saxena A, Tang D, de Hoon M, Anelli V, Mione M, Carninci P, d'Adda di Fagagna F: Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature 488(7410): 231-235, 2012. DOI: 10.1038/nature11179

44 Fragkos M, Barra V, Egger T, Bordignon B, Lemacon D, Naim V, Coquelle A: Dicer prevents genome instability in response to replication stress. Oncotarget 10(43): 4407-4423, 2019. DOI: 10.18632/oncotarget.27034

45 Nojima T, Tellier M, Foxwell J, Ribeiro de Almeida C, Tan-Wong SM, Dhir S, Dujardin G, Dhir A, Murphy S, Proudfoot NJ: Deregulated expression of mammalian lncRNA through loss of SPT6 induces R-loop formation, replication stress, and cellular senescence. Mol Cell 72(6): 970-984.e7, 2018. DOI: 10.1016/j.molcel.2018.10.011

46 Giunta S, Hervé S, White RR, Wilhelm T, Dumont M, Scelfo A, Gamba R, Wong CK, Rancati G, Smogorzewska A, Funabiki H, Fachinetti D: CENP-A chromatin prevents replication stress at centromeres to avoid structural aneuploidy. Proc Natl Acad Sci USA 118(10): e2015634118, 2021. DOI: 10.1073/pnas.2015634118

47 Wang L, Li J, Zhou H, Zhang W, Gao J, Zheng P: A novel lncRNA Discn fine-tunes replication protein A (RPA) availability to promote genomic stability. Nat Commun 12(1): 5572, 2021. DOI: 10.1038/s41467-021-25827-6

48 Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, Reynaud D, Alvarez S, Diolaiti ME, Ugarte F, Forsberg EC, Le Beau MM, Stohr BA, Méndez J, Morrison CG, Passegué E: Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature 512(7513): 198-202, 2014. DOI: 10.1038/nature13619

49 Ahuja AK, Jodkowska K, Teloni F, Bizard AH, Zellweger R, Herrador R, Ortega S, Hickson ID, Altmeyer M, Mendez J, Lopes M: A short G1 phase imposes constitutive replication stress and fork remodelling in mouse embryonic stem cells. Nat Commun 7: 10660, 2016. DOI: 10.1038/ncomms10660

50 Vallabhaneni H, Lynch PJ, Chen G, Park K, Liu Y, Goehe R, Mallon BS, Boehm M, Hursh DA: High basal levels of γH2AX in human induced pluripotent stem cells are linked to replication-associated DNA damage and repair. Stem Cells 36(10): 1501-1513, 2018. DOI: 10.1002/stem.2861

51 Lyu X, Chastain M, Chai W: Genome-wide mapping and profiling of γH2AX binding hotspots in response to different

replication stress inducers. BMC Genomics 20(1): 579, 2019. DOI: 10.1186/s12864-019-5934-4
52 Lam FC, Kong YW, Huang Q, Vu Han TL, Maffa AD, Kasper EM, Yaffe MB: BRD4 prevents the accumulation of R-loops and protects against transcription-replication collision events and DNA damage. Nat Commun 11(1): 4083, 2020. DOI: 10.1038/s41467-020-17503-y
53 Meyer B, Voss KO, Tobias F, Jakob B, Durante M, Taucher-Scholz G: Clustered DNA damage induces pan-nuclear H2AX phosphorylation mediated by ATM and DNA-PK. Nucleic Acids Res 41(12): 6109-6118, 2013. DOI: 10.1093/nar/gkt304
54 Katsube T, Mori M, Tsuji H, Shiomi T, Wang B, Liu Q, Nenoi M, Onoda M: Most hydrogen peroxide-induced histone H2AX phosphorylation is mediated by ATR and is not dependent on DNA double-strand breaks. J Biochem 156(2): 85-95, 2014. DOI: 10.1093/jb/mvu021
55 Chen BR, Quinet A, Byrum AK, Jackson J, Berti M, Thangavel S, Bredemeyer AL, Hindi I, Mosammaparast N, Tyler JK, Vindigni A, Sleckman BP: XLF and H2AX function in series to promote replication fork stability. J Cell Biol 218(7): 2113-2123, 2019. DOI: 10.1083/jcb.201808134
56 Moeglin E, Desplancq D, Conic S, Oulad-Abdelghani M, Stoessel A, Chiper M, Vigneron M, Didier P, Tora L, Weiss E: Uniform widespread nuclear phosphorylation of histone H2AX is an indicator of lethal DNA replication stress. Cancers (Basel) 11(3): 355, 2019. DOI: 10.3390/cancers11030355
57 Groth A, Rocha W, Verreault A, Almouzni G: Chromatin challenges during DNA replication and repair. Cell 128(4): 721-733, 2007. DOI: 10.1016/j.cell.2007.01.030
58 Singh M, Hunt CR, Pandita RK, Kumar R, Yang CR, Horikoshi N, Bachoo R, Serag S, Story MD, Shay JW, Powell SN, Gupta A, Jeffery J, Pandita S, Chen BP, Deckbar D, Löbrich M, Yang Q, Khanna KK, Worman HJ, Pandita TK: Lamin A/C depletion enhances DNA damage-induced stalled replication fork arrest. Mol Cell Biol 33(6): 1210-1222, 2013. DOI: 10.1128/MCB.01676-12
59 Kim J, Sturgill D, Sebastian R, Khurana S, Tran AD, Edwards GB, Kruswick A, Burkett S, Hosogane EK, Hannon WW, Weyemi U, Bonner WM, Luger K, Oberdoerffer P: Replication stress shapes a protective chromatin environment across fragile genomic regions. Mol Cell 69(1): 36-47.e7, 2018. DOI: 10.1016/j.molcel.2017.11.021
60 Seo J, Kim K, Chang DY, Kang HB, Shin EC, Kwon J, Choi JK: Genome-wide reorganization of histone H2AX toward particular fragile sites on cell activation. Nucleic Acids Res 42(2): 1016-1025, 2014. DOI: 10.1093/nar/gkt951
61 Dobersch S, Rubio K, Singh I, Günther S, Graumann J, Cordero J, Castillo-Negrete R, Huynh MB, Mehta A, Braubach P, Cabrera-Fuentes H, Bernhagen J, Chao CM, Bellusci S, Günther A, Preissner KT, Kugel S, Dobreva G, Wygrecka M, Braun T, Papy-Garcia D, Barreto G: Positioning of nucleosomes containing γ-H2AX precedes active DNA demethylation and transcription initiation. Nat Commun 12(1): 1072, 2021. DOI: 10.1038/s41467-021-21227-y
62 Halazonetis TD, Gorgoulis VG, Bartek J: An oncogene-induced DNA damage model for cancer development. Science 319(5868): 1352-1355, 2008. DOI: 10.1126/science.1140735
63 Macheret M, Halazonetis TD: Intragenic origins due to short G1 phases underlie oncogene-induced DNA replication stress. Nature 555(7694): 112-116, 2018. DOI: 10.1038/nature25507
64 Yang Y, Gao Y, Mutter-Rottmayer L, Zlatanou A, Durando M, Ding W, Wyatt D, Ramsden D, Tanoue Y, Tateishi S, Vaziri C: DNA repair factor RAD18 and DNA polymerase Polκ confer tolerance of oncogenic DNA replication stress. J Cell Biol 216(10): 3097-3115, 2017. DOI: 10.1083/jcb.201702006
65 McGrail DJ, Lin CCJ, Dai H, Mo W, Li Y, Stephan C, Davies P, Lu Z, Mills GB, Lee J-S, Lin S-Y: Defective replication stress response is inherently linked to the cancer stem cell phenotype. Cell Rep 23(7): 2095-2106, 2018. DOI: 10.1016/j.celrep.2018.04.068
66 Xing M, Zhang F, Liao H, Chen S, Che L, Wang X, Bao Z, Ji F, Chen G, Zhang H, Li W, Chen Z, Liu Y, Hickson ID, Shen H, Ying S: Replication stress induces ATR/CHK1-dependent nonrandom segregation of damaged chromosomes. Mol Cell 78(4): 714-724.e5, 2020. DOI: 10.1016/j.molcel.2020.04.005
67 Guerrero Llobet S, Bhattacharya A, Everts M, Kok K, van der Vegt B, Fehrmann RSN, van Vugt MATM: An mRNA expression-based signature for oncogene-induced replication-stress. Oncogene 41(8): 1216-1224, 2022. DOI: 10.1038/s41388-021-02162-0
68 Tamayo-Orrego L, Gallo D, Racicot F, Bemmo A, Mohan S, Ho B, Salameh S, Hoang T, Jackson AP, Brown GW, Charron F: Sonic hedgehog accelerates DNA replication to cause replication stress promoting cancer initiation in medulloblastoma. Nat Cancer 1(8): 840-854, 2020. DOI: 10.1038/s43018-020-0094-7
69 Gorgoulis VG, Vassiliou LF, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, Venere M, Ditullio RA, Kastrinakis NG, Levy B, Kletsas D, Yoneta A, Herlyn M, Kittas C, Halazonetis TD: Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434(7035): 907-913, 2005. DOI: 10.1038/nature03485
70 Ren L, Chen L, Wu W, Garribba L, Tian H, Liu Z, Vogel I, Li C, Hickson ID, Liu Y: Potential biomarkers of DNA replication stress in cancer. Oncotarget 8(23): 36996-37008, 2017. DOI: 10.18632/oncotarget.16940
71 Meessen S, Najjar G, Azoitei A, Iben S, Bolenz C, Günes C: A comparative assessment of replication stress markers in the context of telomerase. Cancers (Basel) 14(9): 2205, 2022. DOI: 10.3390/cancers14092205
72 Rahmanian N, Shokrzadeh M, Eskandani M: Recent advances in γH2AX biomarker-based genotoxicity assays: A marker of DNA damage and repair. DNA Repair (Amst) 108: 103243, 2021. DOI: 10.1016/j.dnarep.2021.103243

Received July 28, 2023
Revised September 8, 2023
Accepted September 11, 2023