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Review

DNA replication and cancer: From dysfunctional replication origin activities to therapeutic opportunities

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ABSTRACT

A dividing cell has to duplicate its DNA precisely once during the cell cycle to preserve genome integrity avoiding the accumulation of genetic aberrations that promote diseases such as cancer. A large number of endogenous impacts can challenge DNA replication and cells harbor a battery of pathways to promote genome integrity during DNA replication. This includes suppressing new replication origin firing, stabilization of replicating forks, and the safe restart of forks to prevent any loss of genetic information. Here, we describe mechanisms by which oncogenes can interfere with DNA replication thereby causing DNA replication stress and genome instability. Further, we describe cellular and systemic responses to these insults with a focus on DNA replication restart pathways. Finally, we discuss the therapeutic potential of exploiting intrinsic replicative stress in cancer cells for targeted therapy.

1. Introduction

DNA replication is a crucial biological process that is tightly regulated to ensure accurate and complete transmission of genetic information to daughter cells. The faithful replication of the genome is frequently challenged through stresses originating exogenously or endogenously (described in Table 1). Furthermore, a large number of factors are required for faithful replication, and aberrant function of these factors also pose a threat to accurate replication. Collectively, these challenges can lead to "replicative stress" (RS) that is defined as a transient slowing or stalling of the replication fork [1,2]. The molecular mechanisms underlying the response to RS are not fully understood, however, it is well established that cells employ a complex network of mechanisms and signaling pathways to monitor and deal appropriately with this stress. The existence of replicative stress is widely recognized as a major source of genomic instability as it can lead to replication fork collapse and DNA double strand breaks (DSBs). Furthermore, RS dependent genomic instability can be a consequence of cells entering mitosis with incompletely replicated DNA [1]. Notably, RS is induced by oncogene activity, and it has been described as a hallmark in early stages of tumourigenesis where it serves as a barrier in tumor development [3–7]. The idea that RS is of more than conceptual relevance to the clinic is emerging. For example, genes involved in DNA synthesis that are deregulated in cancer cells may be used as prognostic factors, such as the specialized polymerase Pol theta that is overexpressed in breast cancer [8,9]. Furthermore, several studies suggest taking advantage of RS for cancer therapy where factors in RS pathways could be drug targets [10]. In this regard, the presence of RS in cancer cells may be viewed as an Achilles heel, as this aspect leaves cancer cells sensitive to targeted elimination of pathways promoting RS tolerance with little impact on normal cells of the body.

The replication process is regulated at multiple levels to ensure precise temporal control over the key events. First, pre-replication complexes (pre-RCs) assemble at thousands of DNA replication origins in late mitosis and G1 phase of the cell cycle. This is followed by replication initiation at the G1–S phase transition, where the DNA strand is melted by the MCM helicase and replicative polymerases precisely copy the DNA. To avoid over-replication of the genome, pre-RCs formation is strictly inhibited beyond G1 phase and only a subset of origins are activated during S phase. When replication is finished, cells are in G2 phase where MCM helicase and DNA polymerases are unloaded from DNA. Recent insights have shed considerable light on how the replication processes can be challenged leading to replication stress (see below). However, certain regions of the genome, termed common fragile sites, are particularly difficult to replicate. They are sensitive readouts for replication stress because they are prone to DSBs following RS [1,11]. In this review, we first discuss how DNA replication can be deregulated in cancer cell particularly at the level of replication initiation. Next, we consider cellular mechanisms dealing with such replicative stress

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Table 1
Sources of DNA replication stress and consequences for DNA replication. A number of impediments can slow or stall DNA replication forks leading to replication stress. The Table indicates main sources of stress as well as consequences on DNA replication.

Causes of replicative stress	Consequences on DNA replication
Oncogene induced stress	Aberrant initiation: Uncoordinated origin firing
Secondary structures (such as G-quadruplexes)	Obstacles for replicative DNA polymerase
Repetitive DNA sequences (such as microsatellites)	Slow DNA replication fork Slippage of replication machinery
Collision with Transcription machinery	Obstacles for replicative DNA polymerase
Limiting nucleotides	Slow DNA replication fork
DNA lesions due to exogenous agents	Obstacles for replicative DNA polymerase

as well as diseases associated with the deregulation of these mechanisms. Finally, we outline new therapeutic strategies that enhance replicative stress with potential to improve cancer treatment possibilities.

2. Replication stress as a consequence of deregulated replication initiation

2.1. Introduction

Replication initiation must be strictly coordinated with the cell cycle to ensure faithful duplication of the genome. Pathways that regulate replication initiation are often deregulated in cancer and can contribute to tumourgenesis. To ensure that DNA replication occurs exactly once during the cell cycle the two steps of replication initiation need to be temporarily separated. First, the replicative DNA helicase minichromosome maintenance complex 2–7 (MCM2–7) is loaded at replication origins exclusively during late mitosis and G1 phase, when CDK activity is low [12–14]. This process is termed origin licensing or Pre-RC formation and requires the six-subunit origin recognition complex (ORC) and the activities of the cell division cycle 6 (CDC6) ATPase and the chromatin licensing and DNA replication factor 1 (CDT1).

At the G1/S transition, two kinases, CDK and DBF4-dependent kinase (DDK), activate the MCM helicase, which requires the recruitment of CDC45 and the GINS complex to form the CMG complex [15–17]. The conversion of pre-RCs into bidirectional replisomes requires additional factors, including RecQL4, Treslin/TICRR, MTBP, MCM10 and TopBP1 [15–17]. However, CDKs not only trigger origin firing, they additionally inhibit licensing of newly replicated DNA until the next cycle, which is critical to prevent DNA re-replication (see below). High CDK activity is maintained through G2 phase and mitosis until, ultimately, Cyclin B/CDK1 promotes its own inactivation by activating the APC/C, which triggers mitotic exit and the start of the low CDK period of the next cell cycle that facilitates origin licensing [18]. Once cells enter S phase, a temporal program regulates origin firing to secure that all origins do not fire simultaneously at the onset of S phase, but rather throughout the S phase (reviewed in [19]). This program is likely imposed by several means including the accessibility of limiting replication factors such as CDC45 to chromatin domains [20].

The number of replication origins that are licensed in the G1 phase in a given cell is greater than the number of origins that are activated during an unperturbed S phase (reviewed in [21,22]). Indeed, under normal conditions, only ~10% of replications origins are used while the others remain dormant and are passively replicated. Unperturbed replication is therefore possible when protein levels of the MCM2-7 complex are significantly reduced. However, activation of excess or dormant origins is required to complete replication in situations where replication fork stalling and collapse occurs [23,24]. Although the exact mechanisms underlying dormant origin regulation are unresolved, the two kinases CHK1 and ATR are regulating this process [25–27]. Studies suggest that after its activation following fork stalling, ATR may promote dormant origin initiation via the phosphorylation of MCM2-7 [26]. If origin licensing during G1 phase is severely impaired, however, S-phase entry is prevented by the licensing checkpoint [28,29]. How pre-RC assembly is monitored by this checkpoint and how this signal is transduced to downstream targets is still unclear. However, the licensing checkpoint can be activated when the loading of MCM2-7 onto DNA is severely reduced by various means [30–32]. Importantly, many cancer cells are defective in the licensing checkpoint and can enter S phase despite a severe reduction in the number of origins that have been licensed [28,30,32,33], a feature that may contribute to the genetic instability typically detected in cancer cells. Furthermore, mutations in genes that encode components of the pre-RC such as ORC1, ORC4, ORC6, CDT1 and CDC6 have been identified in patients with the genetic disorder Meier-Gorlin syndrome [34–37]. Cells from these patients display delayed G1 progression, most likely due to the activation of the licensing checkpoint, providing evidence that the licensing checkpoint is operational in humans.

2.2. Deregulated replication initiation in cancer

2.2.1. Too little licensing

In tissue culture systems, reduced licensing appears to mainly challenge cells exposed to RS. Thus, partial depletion of MCM2–7 is tolerated in normal conditions but gives rise to DNA breakage in the presence of replication inhibitors such as hydroxyurea or aphidicolin that cause replication fork stalling and collapse in human cells [38,39]. Similarly, DNA breaks have been observed in MCM2–7 hypomorphic mice, in which dormant origins are reduced due to a lower concentration of MCM2–7 [40]. DNA damage under these conditions can be a consequence of cells entering mitosis with incompletely replicated DNA [40]. The presence of replication intermediates will prevent efficient chromosome segregation and cause chromosome breakage during mitosis [41]. Accordingly, mitotic abnormalities such as lagging chromosomes, anaphase bridges as well as micronuclei have been observed in response to licensing inhibition [40]. Importantly, overexpression of the Cyclin E, which occurs in multiple cancer types [42], impairs MCM2–7 binding to chromatin during G1 and results in cells entering S phase with a reduced number of licensed origins [43] (Fig. 1). Reduced origin licensing can therefore be one of the mechanisms underlying oncogene-induced replication stress preventing the completion of replication and promoting tumourigenesis.

2.2.2. Uncoordinated origin firing

Uncoordinated origin firing occurs in response to oncogene activation and can cause replicative stress and genome instability. Oncogenic c-Myc stimulates aberrant origins firing [44]. C-Myc binds DNA close to replication origins, interacts with members of the pre-RC and co-localizes with replication foci in early S phase, suggesting that c-Myc can directly activate origins independent of c-Myc transcriptional function. Similarly, a transient increase in the level of Cdc45 causes increased origin firing and DNA damage in Xenopus and in human cells [20,45]. The uncontrolled origin activity can exhaust substrates required for replication, such as deoxynucleoside triphosphates (dNTPs) and RPA causing replication fork collapse and DNA damage [46,47].

Overexpression of several oncogenes, such as Cyclin E, HPV E6 and E7 and Ras, reduces inter-origin distances, indicative of increased origin firing [44,47–49] (Fig. 1). Alteration in cyclin-