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Review

Amplifying tumour-specific replication lesions by DNA repair inhibitors – A new era in targeted cancer therapy

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ABSTRACT

Many anti-cancer drugs used in the clinic today damage DNA, resulting in cell death either directly or following DNA replication. Many anti-cancer drugs are exclusively toxic to replicating cells and toxic lesions are formed when a replication fork encounters a damaged DNA template. Recent work shows that replication lesions, similar to those produced during anti-cancer therapy, are commonly associated with cancer aetiology. DNA replication lesions are present in cancer cells owing to oncogene expression, hypoxia or defects in the DNA damage response or DNA repair. Here, I review how novel therapies can exploit endogenous replication lesions in cancer cells and convert them to toxic lesions. The aim of these therapies is to produce similar lesions to those produced by DNA damaging anti-cancer drugs. The difference is that the lesions will be cancer-specific and produce milder side-effects in non-cancerous cells.

1. Replication blocks and cancer

Obstruction of DNA replication is a major threat to genome integrity and contributes to ageing and cancer. On the other hand, obstructing DNA replication by chemical intervention is an effective strategy to kill replicating cancer cells. Revealing the nature of replication lesions and associated response and repair pathways in mammalian cells is crucial to understanding early events that drive tumourigenesis and to develop novel targeted anti-cancer treatments.

There are several natural obstacles encountered by DNA replication forks. Unloading of nucleosomes and other chromatin-attached proteins precedes DNA replication and a defect in the rapid removal of protein barriers would seem likely to obstruct efficient DNA replication. It is unknown to what extent defects in chromatin remodelling contribute to spontaneous DNA replication blocks. However, defects in chromatin remodelling are strongly linked to cancer, which is generally believed to be due to epigenetic silencing of genes. However, efficient chromatin remodelling is instrumental for DNA replication[1] and thus, there is a potential link between chromatin remodelling defects in cancer and an increase in DNA replication lesions. Supporting this notion is the observation that depletion of SET8, a histone methylase results in accumulation of replication lesions and cell death.[2,3]

A portion of the DNA is actively transcribed by RNA polymerases, which will also present a barrier for oncoming replication. It has been shown that homologous recombination may be a pathway to bypass actively transcribed genes in mammalian cells.[4]

Thousands of DNA single-strand breaks (SSBs) are generated in cells each day by reactive oxygen species or following

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processing of base lesions. A SSB that is not repaired before it is encountered by the oncoming replication fork will be converted into a one-ended DNA double-strand break (DSB) and collapse the fork. Homologous recombination repair is triggered at collapsed forks to reinitiate replication.[5–7] The process to repair one-sided DSBs and reinitiate replication is often referred to as break-induced replication. The synthetic lethality between recombination genes and genes involved in oxidative stress response and SSB repair (e.g. TSA1, SOD1, LYS7, SKN7, YAP1 and PARP1)[8,9] underscore the importance of recombination in repair of SSB intermediate lesions that collapse replication forks.

Many cancer therapies exploit replication obstacles to enable clinicians to preferentially kill tumour cells. Some of the most successful anti-cancer drugs (e.g. cisplatin, mitomycin C, melphalan, cyclophosphamide, etoposide, 5-flourouracil (5-FU) and camptothecins) interfere with DNA replication by directly damaging DNA (as with alkylating agents) or by interfering with nuclear metabolism (e.g. by interfering with nucleotide synthesis or topoisomerase function). The accumulation of DNA damage is related to the therapeutic effects of these agents. However, there is considerable heterogeneity in the types of damage caused by these agents and the cellular response to them. For example, 5-FU, which exerts its effects through inhibition of thymidylate synthetase, is an effective inducer of DSBs and cell death[10] whilst other agents that interfere with nuclear metabolism induce cell senescence at therapeutically relevant doses.[11] Although therapies employing these agents are widely used and often effective, our understanding of the lesions formed at the damaged replication forks and the cellular responses to them is surprisingly incomplete.

2. Oncogenes induce replication lesions similar to those produced by anti-cancer drugs

The transformation of a normal cell into cancer is often initiated by oncogene activation, which regularly provides growth signals for proliferation.[12] Oncogene-induced growth signals often mimic stimulatory signals as those transmitted via receptors and transfer cells from quiescent into proliferative state. These precancerous cells are often recognised by gatekeeper proteins early during neoplastic transformation, which activates a tumour barrier.[13] The tumour barrier stops cell proliferation and initiates apoptosis or senescence.[14,15] Gatekeeper proteins that enforce the tumour barrier have been known for decades and the genes encoding these proteins are often mutated in cancers, e.g. *p53*, *Chk2* and *p14*. Many of the gatekeeper proteins have, besides their role in stopping cancer outgrowth, a role in the response to DNA damage and promote cell cycle arrest, apoptosis, senescence and DNA repair.[16] A more comprehensive picture has emerged recently and shows that oncogenes themselves are key activators of the tumour barrier.[17,18] Oncogene expression causes inappropriate DNA replication and is associated with DNA replication lesions.[19–21] Thus, replication-associated lesions may not only be relevant for treatment of cancer, but may also play an important role during tumour development. Several oncogenes (i.e. cyclin E, ras, cdc6, mos and c-myc) cause replication-associated DSB formation, suggesting that the hyper-replication state induced by the oncogenes also triggers replication collapse.[19–21] The mechanisms underlying the induction of replication stress by oncogenes remain elusive. However, overexpression of cyclin E results in an increase in the sites of replication termination that may be related to possible termination problems,[19] whilst ras overexpression is associated with asymmetric replication and re-firing of replication origins.[20] Together these findings suggest that oncogenes may differentially disturb replication progression. It is unclear how wide-spread oncogene-induced replication lesions are in early neoplastic disease, and what their impact on tumour progression is. So far, most tumour types have been shown to display oncogene-induced replication stress, with the exception of testicular germ cell tumours.[22]

Some early molecular changes in cancer may indirectly affect oncogene-induced replication stress. The recent report that the phenotypic effects of mutation of the *Adenomatous polyposis coli* (*APC*) tumour suppressor gene on intestinal crypts (including hyperproliferation of intestinal crypt cells) could be rescued in *APC* mice by *Myc* deletion establishes the c-myc oncogene as the critical mediator of the early stages of neoplasia following *APC* loss.[23] Thus, inactivation of tumour suppressors without apparent relevance to DNA replication may indirectly result in oncogene expression and aberrant replication.

As genes responsible for the checkpoint activation are often mutated during cancer development,[24] cells will evade cell death and continue to proliferate even in the presence of oncogene-induced replication lesions. Combined with a defective DNA repair pathway this would result in error-prone repair of replication lesions. This in turn would contribute to genetic instability,[25] thus inducing further genetic changes required for continuing transformation to malignant phenotypes[26] (Fig. 1).

An amenable strategy for targeted tumour therapy would be to inhibit the normal repair of oncogene-induced replication lesions, which would result in the formation of fatal replication lesions that would kill the tumours. This is likely to show fewer side-effects than current chemotherapy, as only tumour cells exhibit replication-specific lesions.

3. Hypoxia-associated replication stress

More advanced cancers are exposed to another source of replication stress, owing to the tumour microenvironment. Tumours are often hypoxic, which has been shown to disrupt DNA synthesis.[27] These conditions cause replication lesions that activate the ATM and ATR-mediated checkpoint response.[28–30] In spite of increased amounts of replication lesions, DNA repair is down-regulated in hypoxic cells,[31] which cumulatively contributes to the genetic instability observed in these cells.[32,33] To date, it is not clear whether the reduced DNA repair capacity in hypoxic cells contributes to an increase in replication lesions. There is still a need for more research into how hypoxia affects replication and formation of replication lesions. When it comes to potential treatments exploiting replication lesions in hypoxia, inhibitors of the checkpoint response are likely to be more efficient than inhibitors of DNA repair, given the reduced DNA repair levels in these cells.[34]

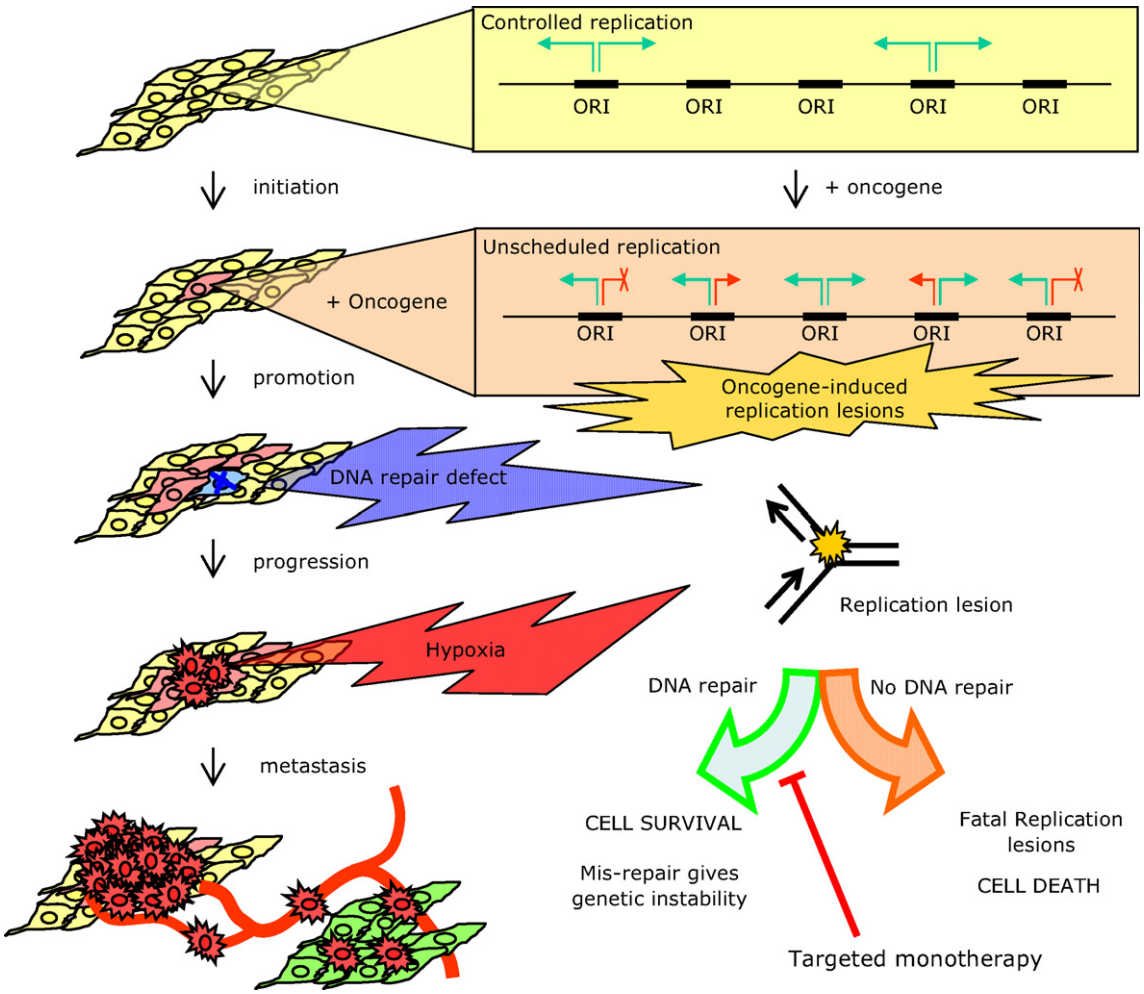

Fig. 1 – Cancer-specific replication lesions as a target for selective cancer treatment. Endogenous cancer-specific replication lesions are present in cancer cells following oncogene expression, DNA repair or damage response defects or during hypoxia. Collapsed replication forks need to be repaired to allow cell survival and continued tumour growth. Tumour cells often have defects in the accurate repair of replication lesions, which will result in illegitimate repair and genetic instability that will drive future mutations. Here, we suggest that tumour-specific replication lesions can be converted into fatal replication lesions through inhibition of DNA repair. Such therapy is likely to be tumour specific as only the cancer cells exhibit replication stress.

4. Replication lesions owing to DNA repair defects in cancer

Inherited mutations in DNA repair genes are often associated with an increased cancer risk. For instance, inherited defects in mismatch repair predispose carriers to hereditary non-polyposis colon cancer[35] and an inherited defect in homologous recombination predisposes carriers to breast and ovarian cancer[36] (see [37] for a list of DNA repair genes associated with cancer). The DNA repair defect is often associated with an increase in mutation rates or gene rearrangements that contribute to genetic instability, which accelerates further genetic changes and progresses cancer development. It has been proposed that genetic instability in cancer can explain all the mutations required to develop a malignant tumour.[38]

There are several different ways a replication fork may be repaired after encountering a replication block and loss of any such repair pathway may contribute to an increased amount of replication lesions. The most efficient way of preventing replication damage is to arrest the replication fork and await the removal of the block by DNA repair (Fig. 2A). An alternative to removing the DNA damage is to await the arrival of the opposite replication fork to bypass the lesions (Fig. 2B). Another way is to bypass the replication lesion, which may be achieved in several different ways. In template switching, the opposite nascent DNA strand is used as a template for replication, followed by reinitiation of replication by recombination downstream of the replication block,[7] or by branch migration across the DNA lesions (Fig. 2C). The replication block may also be bypassed by trans-lesion synthesis across the damaged base[39] or possibly by repriming replication downstream of the lesion[40], although the latter has not been demonstrated in mammalian cells. Gaps remaining after lesion bypass are repaired post replication by homologous recombination or trans-lesions synthesis. However, the integrity of arrested replication forks can often not be maintained,

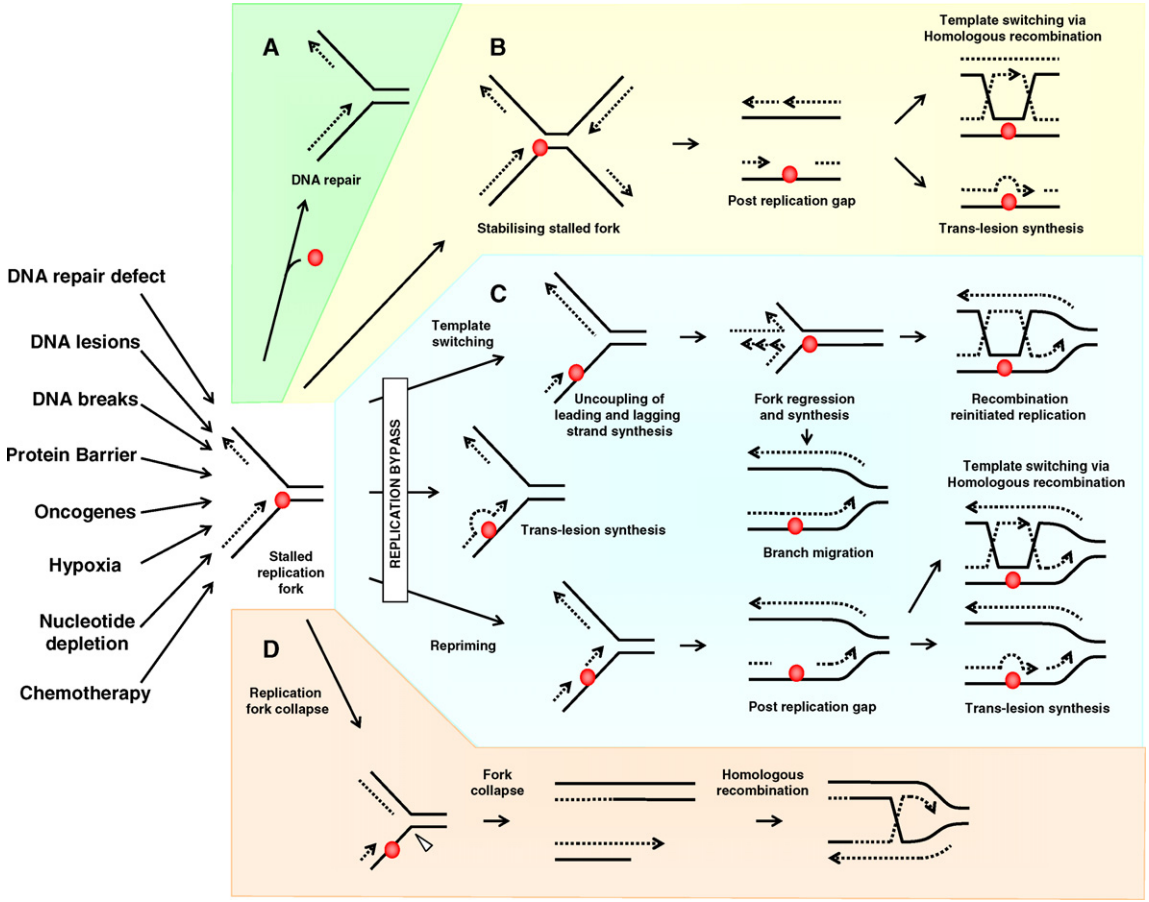

Fig. 2 – Pathways at stalled replication forks. A replication fork stalls when encountering a DNA road block (e.g. breaks, lesions or protein barriers), after depletion of nucleotides, or following oncogene-induced replication stress. (A) The best option is that DNA repair removes the replication block to allow replication progression. (B) If this is not possible, the stalled fork can be stabilised to await the arrival of the opposite replication fork. Remaining gaps may then be filled either through template switching through homologous recombination or bypassing the lesion using trans-lesion synthesis. (C) The stalled fork may also directly bypass the lesion using trans-lesion synthesis or by repriming downstream of the replication block. It may also bypass the lesion by template switching directly at the replication fork. This requires uncoupling of the leading and lagging strand synthesis and regression of the replication fork. The newly synthesised lagging strand is then used as template for the leading strand synthesis and homologous recombination is then initiated at the DNA end to rescue and restart replication. (D) Bypassing or repair of a severe DNA replication block may not be possible. Under these circumstances, the replication fork may, if needed, be cleaved by endonucleases to collapse the replication fork into a DNA double-strand break. The DNA ends can then be trimmed and the block removed to present a clean DNA end that can be used to restart replication using homologous recombination.

which results in replication collapse and DSB formation (Fig. 2D). Homologous recombination is a key repair pathway to reactivate collapsed replication forks in mammalian cells.[7] The importance of homologous recombination in replication repair is illustrated by the lethal phenotype and the formation of DSBs after replication when the RAD51 recombinase is disrupted.[41] Inherited mutations of the BRCA1 and BRCA2 genes predispose to breast or ovarian cancer. Both genes encode proteins involved in homologous recombination repair and loss of this pathway contributes to genetic instability, which is likely to explain the tumour suppressor function of these two proteins.

Replication stalling has been extensively investigated in yeast and mammalian cells using hydroxyurea, which quenches the tyrosine free radical within the M2 subunit of ribonucleotide reductase, thus restricting synthesis of all deoxyribonucleoside triphosphates.[42] In yeast, a proficient checkpoint stabilises the replisome at stalled forks[43,44] to prevent replication collapse.[45] In the absence of *rad53* replication forks reverse and extensive single-stranded DNA regions are formed[46] as well as sister chromatid junctions, structures that may facilitate replication restart. It has been shown that the replication checkpoints prevent aberrant recombination at stalled replication forks in yeast.[47] The response to hydroxyurea is somewhat different in mammalian cells. Hydroxyurea collapses replication forks into replication-associated DNA double-strand breaks (DSBs), in the presence of functional replication checkpoints.[6] It has been shown that the collapse of stalled replication forks is mediated by endonucleases such as Mus81.[48] Furthermore, homologous

recombination at hydroxyurea-stalled replication forks is not suppressed by replication checkpoints, in fact, the Chk1 pathway is required to activate recombination repair of hydroxyurea-induced replication associated DSBs.[49] A similarity between mammals and yeast is that ATR-Chk1 as well as p53 suppresses the extent of replication collapse after hydroxyurea treatment.[49,50] Furthermore, several recent reports show that the ATR-Chk1 signalling pathway controls the induction of apoptosis in replication-stressed S-phase cells.[51–53] Altogether, it appears so far that the ATR-mediated DNA replication checkpoint, in mammals plays a more important role in preventing DNA replication firing[54] and stabilising replication forks to control a fine balance between recombination mediated rescue of replication forks and cell death.

5. Targeting replication stress for cancer treatment

Replication lesions caused by oncogene activation, hypoxia or loss of DNA repair include DSBs at replication forks and other lesions that resemble those produced by anti-cancer treatments.[19,55] It is critical that these lesions are repaired for the cancer cells to survive. Here, I propose that DNA repair inhibitors can be used to make existing endogenous cancer-specific replication lesions more toxic, resulting in fatal replication lesions to selectively kill cancer cells (Fig. 1). The nature of replication lesions produced following chemotherapy or oncogene-induced stress is poorly understood. Although there are several replication repair pathways identified, we currently have little information regarding their complex interplay. Indeed, more intense basic research is required in this area to identify novel anti-cancer targets.

Other groups and Mine have shown that DNA repair inhibitors work as single agents to treat cancer, particularly in DNA repair defective tumours. A portion of inherited breast and ovarian cancers lack a wild-type copy of the *BRCA1* or *BRCA2* genes, resulting in cells that are defective in HR repair[56,57] and show extensive replication-associated lesions.[55,58] We showed that these recombination defective cells are 1000-fold more sensitive to PARP inhibitors used in monotherapy than are the heterozygote or the wild-type cell lines, showing the potential for exploitation to specifically treat *BRCA1* or *BRCA2* defective tumours.[8,59] PARP inhibitors are currently in phase II clinical trials in cancer patients carrying *BRCA1* or *BRCA2* mutations.[37] A suggested explanation for the extreme sensitivity of these cells following PARP inhibition is that homologous recombination at replication forks serves as a back-up repair pathway for PARP-1 dependent SSB repair.[8,60–62] However, the exquisite sensitivity may also be explained by other activities of PARP which may relate to the repair of the increased amount of replication lesions present in homologous recombination defective cells.

6. Conclusions

It is increasingly appreciated that stresses generated at replication forks offer many opportunities for novel targeted therapies for cancer. Tumour cells suffer from many obstacles to replication often resulting in the chronic activation of checkpoints.[18] Drugs designed to upset the checkpoint responses to these obstacles might tip the fine balance in favour of tumour cell destruction. Furthermore, oncogene-induced replication lesions can potentially be further exploited for cancer treatment. If DNA lesions produced at the early stages of tumour development are amplified by inhibiting DNA repair or replication bypass, an increase in oncogene-induced replication collapse could be expected. Such replication collapse may resemble that achieved during anti-cancer treatments and result in cell killing.[8,9] A future challenge will be to achieve better understanding of the replication lesions produced by oncogenes and their repair, as it will be important to only target the pathways used to repair the oncogene-induced lesions.

Conflict of interest statement

None declared.

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