DNA replication-associated lesions: importance in early tumorigenesis and cancer therapy

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Abstract

DNA lesions resulting from impaired progression of replication forks are implicated in genetic instability and tumorigenesis. Because the cellular response to these lesions poses an important tumorigenesis barrier, the responsible signalling and repair pathways are often mutated or inactive in tumours. Here, we discuss how such deficiencies can in turn be exploited for cancer therapy.

Replication-associated lesions

During every round of DNA replication, moving replication forks encounter countless obstacles and lesions on the DNA template, such as DNA-bound proteins, difficult to replicate secondary structures or unrepaired DNA damage [1]. This can result in prolonged stalling of the fork and, for example after collision with unrepaired SSBs (single-strand breaks), fork collapse and the generation of lethal DNA DSBs (double-strand breaks) [2]. Such replication-associated lesions are implicated in genomic instability and tumorigenesis, but can also be exploited for cancer therapy, especially because cancer cells are highly proliferating and often defective in the DNA damage signalling or DNA repair pathways that deal with these lesions.

Replication-associated lesions in early tumorigenesis

Recent studies suggest the existence of tumorigenesis barriers that slow or inhibit the progression of pre-neoplastic lesions to neoplasia (tumours). One such barrier involves oncogene-induced DNA replication stress. This replication stress leads to activation of DNA damage response pathways involving the ATM (ataxia telangiectasia mutated)–Chk2 (checkpoint kinase 2) and the ATR (ataxia telangiectasia mutated- and Rad3-related)–Chk1 signalling cascades, arrest in S- and G2-phases of the cell cycle and apoptosis. The activation of the DNA damage response precedes genomic instability in tumour development [3,4]. It was observed that overexpression of the oncogene cyclin E, which is involved in origin licensing, leads to altered replication dynamics, formation of single-stranded DNA, phosphorylation of the checkpoint kinase Chk1 and replication-associated DSBs [3,5]. Early pre-cancerous lesions were found to display allelic imbalance at specific chromosomal loci termed ‘common fragile sites’ [4], indicating DNA breakage at these loci, which is normally observed after partial inhibition of replication fork progression [6]. These results suggest that, in pre-cancerous lesions, deregulated origin firing can lead to impaired replication fork progression, which is in turn sensed as replication stress. The exact relationship between origin firing and replication stress requires further investigation.

A second tumorigenesis barrier is mediated by oncogene-induced senescence [7–9]. Recently, a link between these two barriers was shown by demonstrating that senescence induced by overexpression of several oncogenes, including cyclin E, is dependent on the replication stress-induced DNA damage response described above [5]. This suggests the existence of selection for cells with mutated or inactivated DNA damage response pathways for tumour progression. Accordingly, loss of the checkpoint factors 53BP1 and Chk2, in addition to mutant p53, was found in more advanced cancer stages [4]. Loss of checkpoint factors, again, may also lead to perturbed replication dynamics and replication-associated DSBs, which might further increase genomic instability [6]. In addition, cancer cells are very often defective in DNA repair mechanisms that deal with replication-associated DNA damage, such as HR (homologous recombination) repair [10,11]. In the following, we discuss how defects in DNA repair mechanisms can be exploited to amplify endogenous replication-associated lesions for cancer therapy.

Pathways of repairing replication-associated lesions

A major pathway for the repair and restart of collapsed replication forks is HR, a complex pathway that repairs one- or two-ended DSBs, and possibly other damaged fork structures, by utilizing homologous sequences on the sister chromatid [12–14]. The HR factor BRCA2 (breast-cancer susceptibility gene 2) has also been proposed to be involved...
in overcoming replication blocks [15]. HR is regulated by several signalling pathways. For example, the DNA damage response kinase Chk1 interacts with Rad51 and is required for Rad51 foci formation and HR repair of collapsed replication forks [16]. The ATM checkpoint kinase has also been implicated in HR repair of DNA breaks that arise from the collision of replication forks with DNA SSBs [17].

An alternative pathway for the repair of DSBs is NHEJ (non-homologous end joining), which directly rejoins DSB ends by ligation, with little requirement for sequence homology. Although NHEJ is involved in repairing DSBs resulting from fork collapse during S-phase, it plays a less important role than HR [12].

Exploiting replication-associated lesions for therapy

A new concept for cancer therapy is to amplify endogenous tumour-specific DNA lesions, to specifically kill tumour cells. This can be achieved by inhibition of DNA repair. For example, inhibitors of PARP-1 [poly(ADP-ribose) polymerase-1] are widely used to decrease the efficiency of SSB repair. An increased amount of SSBs is likely to lead to more collapsed replication forks that require recombination repair to restart. Indeed, HR has a very important role in PARP-1-defective cells, as inhibition or loss of PARP-1 is associated with a hyper-recombinogenic phenotype as indicated by a high level of sister chromatid exchanges and Rad51 foci [18,19]. Equally, cells defective in recombination may therefore be more sensitive to inhibition of PARP-1.

This idea was put into practice for cells that are mutated in the breast-cancer susceptibility genes BRCA1 or BRCA2, which encode proteins involved in HR repair [10]. Heterozygous carriers of a mutation in either gene have a considerably increased risk of breast or ovarian cancers that arise from cells that have lost the wild-type copy. The loss of HR leads to alterations in DSB repair and thus accelerates genetic instability, which is likely to drive cancer development [10]. Cell lines homozygous for either the BRCA1 or BRCA2 mutation are highly sensitive to PARP inhibitors [20,21]. These cells are 100–1000-fold more sensitive to PARP inhibitors than the heterozygote or the wild-type cell lines, and PARP inhibitors can even induce regression of tumours derived from the homozygous mutated cells. siRNA (small interfering RNA)-mediated depletion of BRCA2 in MCF7 (wild-type p53) and MDA-MB-231 (mutated p53) breast cancer cell lines also results in sensitivity to PARP inhibitor-mediated cytotoxicity [21]. This shows that BRCA2 defective breast cancers can be specifically targeted using inhibitors of PARP-1 alone, a treatment that is likely to be highly tumourspecific since only the tumours (which are BRCA2<sup>+/−</sup>) in the BRCA2<sup>−/−</sup> patients are completely defective in HR repair. The use of an inhibitor of a DNA repair enzyme alone, in the absence of an exogenous DNA-damaging agent, to selectively kill a tumour represents a new concept in cancer treatment. Clinical trials using PARP inhibitors alone are under way [22].

If tumours are not inherently deficient in HR, down-regulation of HR activity in combination with PARP inhibitors could be a suitable therapeutic strategy to increase replication-associated lesions. A variety of proteins involved in HR were recently investigated for their impact on the cytotoxicity of the PARP inhibitors KU0058684 and KU0058948 as proof of principle. RNAi (RNA interference)-mediated silencing of or deficiency in the HR factors Rad51, Rad54, Dss1 and RPA1, the DNA damage signalling proteins ATM, ATR, Chk1, Chk2 and Nbs1 and components of the Fanconi’s anaemia signalling pathway led to increased sensitivity to PARP inhibition [23]. For therapy, small molecule inhibitors are more desirable than RNAi. Rad51 expression can also be down-regulated by inhibiting c-Abl kinase with imatinib mesylate (Gleevec), resulting in cellular sensitization at least to DNA-damaging agents [24]. Inhibitors of Chk1 (e.g. UCN-01) are widely under investigation for cancer therapy. Chk1 activity is not only required for HR repair of collapsed replication forks, but also for maintaining high rates of replication fork progression, via a currently unknown mechanism that is HR-independent but might involve maintenance of the replisome [6,25]. Chk1 inhibition leads to rapid accumulation of replication-associated DNA DSBs [26], which could result from a combination of accumulating replication-associated lesions and inability to repair them by HR. These results highlight the importance of Chk1 in preventing DNA damage during replication, which makes it highly interesting as a potential target for cancer therapy. ATM inhibitors present another possibility of indirectly inhibiting HR. PARP-1<sup>−/−</sup> cells are sensitive to the ATM inhibitor KU55933, and ATM-deficient cells are conversely sensitive to the PARP inhibitor 4-amino-1,8-naphthalamide. Inhibition of PARP leads to ATM activation, and PARP inhibitor-induced HR repair is abolished in ATM-inhibited cells [17].

NHEJ is also targeted for cancer therapy, by using inhibitors of DNA-PK (DNA-dependent protein kinase), a central NHEJ factor. However, it is not clear whether inhibition of NHEJ, which is less important during S-phase than HR, presents a possibility to increase the cytotoxicity of replication-associated lesions, and the combination of DNA-PK and PARP inhibitors did not reduce survival in mouse embryonic fibroblasts [27].

In conclusion, DNA replication-associated lesions are commonly formed during tumorigenesis and can be efficiently exploited for targeted therapy. A future challenge will be to gain further insight into the origins and nature of lesions involved in tumorigenesis and to identify components of DNA damage signalling or DNA repair pathways that are suitable for targeting to specifically kill cancer cells.

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References


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