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## Replication stress

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**Title:**

Never tear us a-PARP: Dealing with DNA lesions during mitosis

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**Abstract**

Tumors defective in homologous recombination (HR) are highly sensitive to poly ADP-ribose polymerase (PARP) inhibition, however the cell biological mechanisms underlying this synthetic lethality remain elusive. We recently identified that PARP inhibitor-induced DNA lesions persist until mitosis, subsequently causing mitotic chromatin bridges, multinucleation and apoptosis. Here, we discuss the implications of these findings.

**Main text**

BRCA1 and BRCA2 are essential in achieving error-free repair of DNA double stranded breaks (DSBs) through homologous recombination (HR). Mutations in HR genes, therefore, result in defective genome maintenance and predispose to tumorigenesis, primarily in breast and ovarian tissues. The resulting HR-defective tumors appeared highly sensitive to inhibitors of PARP,<sup>1,2</sup> resulting in the successful clinical development of PARP inhibitors for patients with BRCA1/2 mutant cancers. Unfortunately, however, patients often develop resistance to PARP inhibitors and relapse. To identify possible new combination strategies that improve PARP inhibitor therapy, molecular insight into the mechanism of PARP inhibitor cytotoxicity in HR-deficient tumor cells is required.

Exactly how cancer cells with defective HR die following PARP inhibition remains incompletely clear. Initially, loss of PARP was reported to cause single stranded DNA break (SSB) accumulation, owing to the role of PARP in base excision repair (BER).<sup>1,2</sup> High levels of SSBs would then, through the course of DNA replication, lead to DSB formation, which in HR-deficient cells ultimately result in cell death. However, the role of PARP inhibitors in BER and ensuing SSB accumulation is debated, suggesting that other functions of PARP and HR genes are involved in cell death induction.<sup>3</sup> Specifically, HR components as well as PARP were found to have important functions in the protection and restart of replication forks.<sup>4,5</sup>

We recently showed that HR-deficient cancer cells indeed have compromised fork stability when treated with PARP inhibitors, and accordingly present with high levels of FANCD2 foci during replication.<sup>6</sup> Surprisingly, these DNA lesions appear to remain unresolved in S or G2 phase, and instead are propagated into mitosis, resulting in chromatin bridge formation in anaphase.<sup>6</sup> Of note, using live cell imaging we observed that PARP inhibitor-induced chromatin bridges led to multinucleation and cell death.

Our findings reiterate that replication lesions can be transmitted into mitosis.<sup>7</sup> Although we focused on the mitotic transmission of PARP inhibitor-induced lesions, it is highly likely that numerous other replication lesions can ultimately be transmitted into mitosis, and through this phenomenon affect cell fate (Fig. 1). In this Author's View, we will further discuss our findings and implications thereof.

### **Consequences and processing of mitotic DNA damage**

Exactly how mitotic DNA damage triggers cell death is unclear. One possibility involves differential wiring of the apoptotic machinery during mitosis, or that apoptosis is activated at a lower threshold following mitotic entry. Indeed, mitotic kinases, notably Cyclin-dependent kinase-1 (Cdk1), were reported to modify pro- as well as anti-apoptotic proteins, including Bcl-X<sub>L</sub>, Bcl-2, Mcl-1 and several caspases.<sup>8</sup> However, multiple mitosis-dependent modifications of the apoptosis machinery make cells resistant rather than susceptible to apoptosis. Additionally, some of the reported pro-apoptotic effects during mitosis are only instigated during prolonged mitotic spindle checkpoint arrest, and therefore do not necessarily reflect the situation of cells entering with unresolved DNA lesions.

Another possibility involves replication-mediated joint DNA molecules being transformed into more toxic DNA lesions, such as DSBs, during mitosis. Indeed, replication-mediated DNA lesions that remain unresolved until mitotic entry are acted upon by the MUS81/EME nuclease,<sup>7</sup> probably as part of a complex of multiple structure-selective nucleases.<sup>9</sup> Processing of DNA lesions does not necessarily lead to an accumulation of toxic DNA structures, since nuclease-mediated processing of mitotic DNA lesions is an initial step in their resolution. However, PARP inhibitor treatment in the context of HR deficiency might lead to an overwhelming load of DNA lesions, beyond the capacity of mitotic repair. Alternatively, efficient processing of mitotic DNA lesions may be hampered in cells with inactivated HR and PARP.

Additionally, physical tension, exerted onto chromatin bridge DNA by spindle force, could be responsible for cell death, either directly or through the generation of DSBs. Alternatively, cytokinesis failure and consequent formation of multinucleated cells might constitute another way cells could eventually generate toxic DNA lesions which lead to cell death.<sup>10</sup>

Likely, multiple of the abovementioned mechanisms occur in parallel to instigate cell death. A notion which is supported by our finding that subsets of PARP inhibitor-treated cells die prior to completing

mitosis, whereas others fail to undergo cytokinesis, leading to multinucleation and subsequent cell death. Understanding apoptotic cues in mitosis will prove pivotal for exploiting this knowledge to potentiate therapeutic strategies relying on mitotic catastrophe for anti-cancer cytotoxicity.

### **The role of mitotic transmission of DNA lesions in cancer cell fate**

Mitotic catastrophe is frequently thought to be responsible, as least partly, for cytotoxicity of current cancer treatments. Our report, to our knowledge, for the first time uses forced mitotic bypass induced by depletion of Early Mitotic Inhibitor-1 (EMI1), as a tool to assess the contribution of mitotic progression to cell death. We implemented this tool to test the contribution of mitotic progression to the cytotoxicity of PARP inhibitors in HR-deficient cells. However, this approach can be applied similarly to test other agents. We showed that, for instance, cisplatin, also induces mitotic chromatin bridges in a HR-deficient cancer cells.<sup>6</sup> Additionally, oncogene-induced replication stress also results in under-replicated lesions, and it would therefore be interesting to see whether mitotic catastrophe is observed in tumors with oncogene-induced replication stress. Above findings would fit a model in which mitotic catastrophe presents a possible mechanism clearing cells upon chromosome mis-segregation, genetic instability and tumorigenesis. Since the cytotoxicity of PARP inhibitors in HR-deficient cells is promoted by mitotic progression, it is interesting to speculate that cancer cells harboring DNA replication lesions maintain viable by arresting the cell cycle. Targeting of DNA damage checkpoint kinases, including WEE1, could be used to abrogate cell cycle arrest, push PARP inhibitor-treated cells into mitosis, and promote cell death.

In summary, a better understanding of the mitotic ‘death cues’ that underlie the cytotoxicity of PARP inhibitors, and possibly many other anti-cancer agents, will aid in directing the development of improved cancer treatments. Although mitotic catastrophe in response to DNA damage induction has been reported for decades, molecular cues that are responsible for mitotic catastrophe remain elusive, and additional research is warranted to uncover the mechanisms underlying this phenomenon.

### **Disclosure of Potential Conflicts of Interest:**

No potential conflicts of interest were disclosed.

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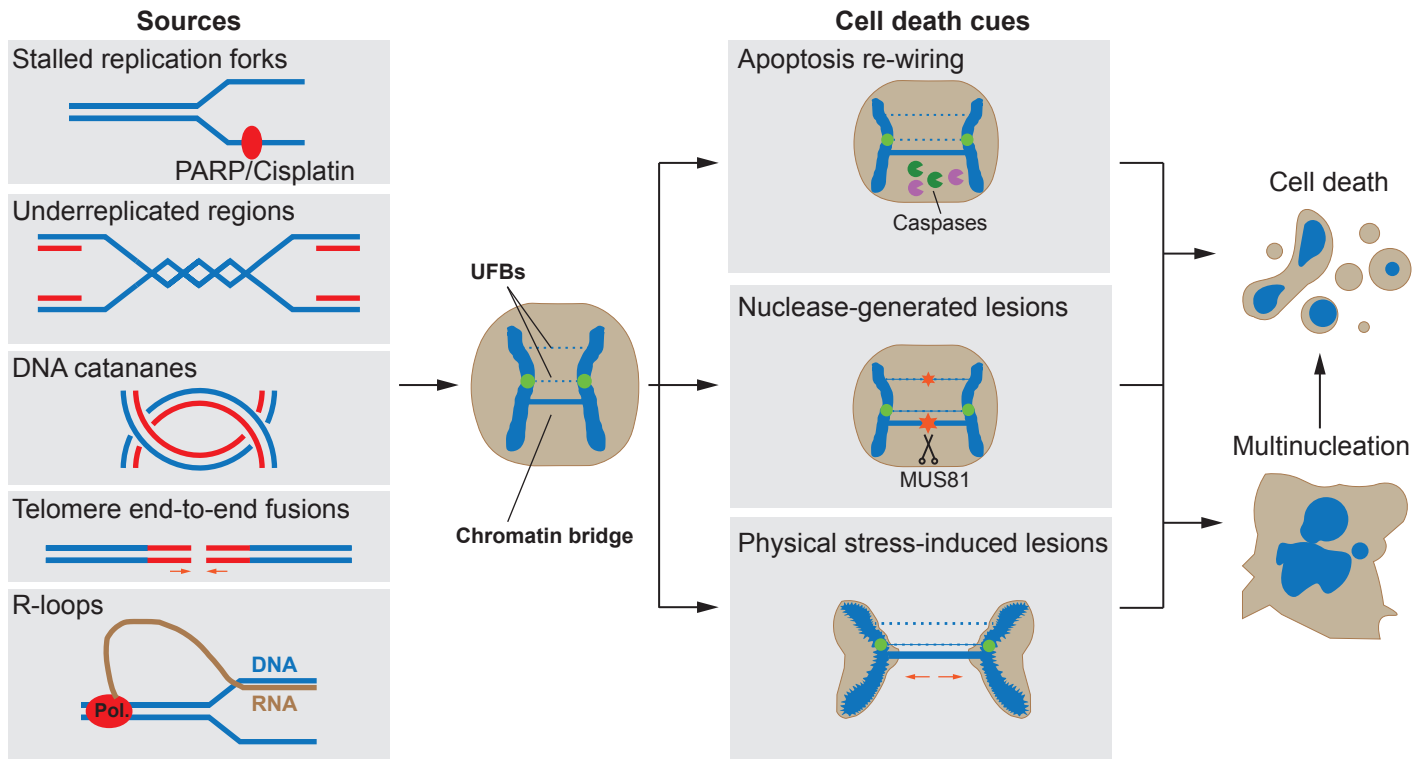
We apologize to colleagues, whose work could not be cited due to space limitations. M.A.T.M.v.V. is supported by grants from the European Research Council (ERC-Consolidator grant 2015-682421), the

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**Figure 1**



**Figure 1.** Dealing with unresolved DNA lesions during mitosis. Treatment of cancer cells with PARP inhibitor and other agents cause replication lesions that frequently enter mitosis, resulting in the formation of Ultra-fine bridges(UFBs) and chromatin bridges. If unresolved, DNA bridges result in multinucleation and cell death. However, the responsible cell death cues are currently unknown.