

## IN THE SPOTLIGHT

## Collateral Genome Instability by DNA Damage in Mitosis

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**Summary:** Chromosome segregation errors and DNA damage are in a vicious cycle in cancer cells. Bakhoum and colleagues show that the molecular response to damaged DNA during mitosis impairs the chromosome segregation machinery, adding a new level to the already dangerous relations between different kinds of genomic instability. *Cancer Discov*; 4(11); 1256–8. ©2014 AACR.

See related article by Bakhoum et al., p. 1281 (7).

A high rate of changes to a cell's genome enables the acquisition and evolution of the well-known hallmarks of cancer. As such, virtually all cancer cells exhibit genomic instability in one form or another. For example, at least two thirds of human cancers are mosaic aneuploid as a result of frequent gains and losses of whole chromosomes during cell divisions. Such whole-chromosomal instability (wCIN) can promote gains of extra copies of oncogenes or losses of tumor-suppressor genes, and it allows selection of karyotypes that thrive in certain environments. Tumor relapse following the initial success of anticancer therapies, as well as anticancer drug resistance, has therefore been attributed to wCIN (1). Although the wCIN phenotype is of benefit to the survival of a tumor cell population, too many mistakes in chromosome segregation are lethal. wCIN must thus be maintained at the right level, and this "weakness" may be exploited in anticancer therapy.

Another form of genomic instability frequently observed in cancer cells is instability at the level of chromosome structure. Structural CIN (sCIN) encompasses a variety of changes to the genome, including translocations, deletions, inversions, and fragmentations. sCIN is caused by poor repair of damaged DNA, due to, for example, mutations in DNA repair pathway components or inefficient cellular responses to DNA damage. The first response after detection of damage is the DNA damage response (DDR) pathway, of which the kinases ATM and CHK2 are crucial components. The DDR signaling network simultaneously initiates repair and halts the cell-cycle machinery to allow more time for repair to take place. This first response also initiates a second, slower, p53-dependent response (2).

Although they were initially viewed as independent processes, evidence is mounting that wCIN and sCIN are linked in a number of ways. Chromosome fusions by telomere damage cause dicentric chromosomes that may subsequently end up in

only one of the daughter cells after mitosis. Another cause of wCIN is replication stress, a term that signifies the response to DNA damage caused by stalled replication forks. The mechanism for this is unknown, but alleviation of replication stress in colorectal cancers that display wCIN reduced chromosome segregation errors in a recent study (3). Conversely, DNA can become damaged as a result of errors in chromosome segregation. The most frequently observed errors in cancer cell lines are chromosomes that lag behind the separating packs of chromosomes at anaphase (4). These laggards can acquire damage during cytokinesis, resulting in deletions and chromosomal translocations in daughter cells (5). These and other types of missegregated chromosomes also form micronuclei, structures often used as a marker in cancer diagnosis. Micronuclei suffer from replication stress and damaged DNA (6). The obvious result of the causal connections between errors in chromosome segregation and DNA damage is a vicious cycle: wCIN can cause DNA damage, which in turn can cause wCIN, and so on and so forth (Fig. 1). It is not difficult to see that such a cycle may contribute in important ways to tumor heterogeneity and rapid evolution of the tumor cell population.

In this issue of *Cancer Discovery*, Bakhoum and colleagues (7) report a surprising novel connection between DNA damage and wCIN. They investigated the consequences of damaging DNA during mitosis and observed an elevated frequency of chromosome missegregations in cancer cells as well as in immortalized normal human cells. The type of missegregation most frequently observed was that of lagging chromosomes. These laggards are the result of hyperstabilization of chromosome-spindle connections and escape detection by the main mitotic cell-cycle checkpoint. Bakhoum and colleagues show that activation of the ATM–CHK2 pathway was responsible for this hyperstabilization, at least in part via the well-known spindle regulators Aurora A and PLK1. Chemical inhibition of ATM, CHK2, Aurora A, or PLK1 prevented not only hyperstability of chromosome-spindle connections but also the increase in lagging chromosomes following mitotic DNA damage. Finally, the authors observed that cell lines most responsive to CHK2 inhibitors (with regard to reducing the frequency of lagging chromosomes) were generally the ones with the highest level of mitotic DDR pathway activation.

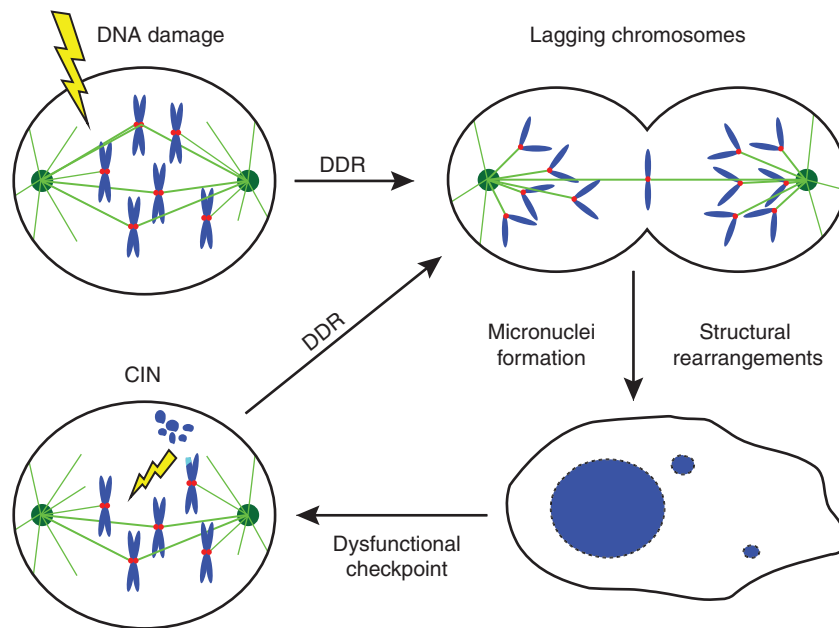
This new study raises a number of questions related to (cancer) cell biology and cancer therapy. From a molecular mechanistic view, it will be of interest to examine how the mitotic

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**Figure 1.** A cycle of genome instability in cancer cells. DNA damage during mitosis activates the DDR, causing lagging chromosomes in anaphase. This can induce sCIN by generation of micronuclei (that undergo replication stress and are susceptible to pulverization) or by structural rearrangements of chromosomes damaged during cytokinesis. Moreover, the initial DNA damage is not repaired in mitosis and persists until G<sub>1</sub>, when double-strand breaks are corrected by the error-prone process of nonhomologous end-joining. If checkpoints fail and damage persists until the next mitosis, the DDR will be active and cause more wCIN, establishing positive feedback between wCIN and sCIN.

DDR affects the factors that regulate stability of chromosome–spindle interactions. Also, seemingly contrary to what is reported here, hyperstability of these interactions and a high level of lagging chromosomes were observed in *CHK2*-knock-out cells, which was attributed to a requirement of *CHK2* and its target *BRCA1* for normal spindle formation (8). How can *CHK2* have opposing effects on the same processes in mitosis? Do spatially or temporally distinct functional pools of *CHK2* exist that either promote spindle formation during normal cell divisions or mediate an initial DDR upon mitotic DNA damage? Given the frequent occurrences of *CHK2* mutations in cancer (8), the mechanistic details of *CHK2*'s roles in chromosome segregation are worth more thorough examination.

Although DNA damage in mitosis elicits an initial DDR, it does not halt cell-cycle progression and cannot activate the repair pathways during mitosis. The observation by Bakhom and colleagues that the triggering of a genome-protection program in mitosis accomplishes the opposite, namely wCIN, raises the question of the purpose of maintaining the DDR during mitosis. The seemingly unfavorable effect may be an unfortunate by-product of the adopted role of ATM–*CHK2* in spindle formation that cells have been unable to uncouple from DDR activation after damage in mitosis. A perhaps more satisfying possibility is that this response in mitosis is not so disadvantageous as it seems at first sight. The *CHK2* ortholog in *Drosophila melanogaster* embryos is essential for a “mitotic catastrophe” signal in response to genotoxic stress to ensure elimination of mutant and aneuploid nuclei (9). Because repair of DNA damage in G<sub>1</sub> phase occurs through the error-prone nonhomologous end-joining pathway, cells may have evolved to senesce or die when faced with extensive damage. In a healthy organism, it might thus be a mechanism to amplify genomic damage to ensure clearance of damaged cells after completion of mitosis.

An exciting implication of the present study is that it provides an explanation for how replication stress can cause wCIN: If DNA damage obtained in interphase persists throughout

mitosis, activation of the initial phases of the DDR will cause hyperstabilization of chromosome–spindle interactions and ensuing errors in chromosome segregation. But the impact may go quite beyond that. In order for cells to proceed with division when faced with damaged DNA, be it from replication stress or other sources, the premitotic cell-cycle checkpoints need to be dysfunctional, for instance by mutations in the p53 pathway. The findings by Bakhom and colleagues provide a mechanism for the acquisition of sCIN and wCIN without the need for prior inactivation of cell-cycle checkpoints. DNA damaged in mitosis does not activate the full DDR beyond ATM–*CHK2*, and the resulting lagging chromosomes do not activate the mitotic checkpoint. Damage obtained in mitosis is thus an interesting candidate for a tumor-initiating event, or at least a very early event in tumor progression, especially if the combined damages and missegregations inactivate the p53 pathway to ensure cell-cycle progression beyond the subsequent G<sub>1</sub> phase. Whether mitotic DNA damage can trigger the vicious cycle, or whether the cycle is permitted to start by prior mutations in genome surveillance pathways, remains to be seen. It may be of interest to examine whether subclones of proliferating wCIN cells can grow out when chromosomes of healthy human cells are damaged in mitosis.

DNA-damaging agents are successful in treatment of a variety of cancers. As mentioned before, wCIN can be beneficial for tumors, as it causes tumor heterogeneity and improves the tumor's ability to constantly evolve and adapt to environmental changes. The findings by Bakhom and colleagues raise the possibility that by enhancing wCIN, DNA-damaging therapeutic agents may accelerate the appearance of resistant subclones. On the other hand, too much CIN is lethal for tumor cells, so DNA-damaging agents could conceivably have higher efficacy on tumors that already display high levels of wCIN. It is of interest to note that agents like 5-FU and oxaliplatin that cause replication stress and DNA damage have long been successful in treating colorectal cancer, of which an estimated 85% is aneuploid (10). In a similar

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vein, DNA-damaging agents may display synergy with anti-mitotic drugs like paclitaxel that kill cells by forcing extensive chromosome missegregations. It will be important to know whether specific cancer types are sensitive to this, and if so whether sensitivity is related to traits such as level of wCIN, and/or endogenous genotoxic stress before drug treatment. Bakhoun and colleagues provide an exciting new lead to further explore these questions.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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### REFERENCES

- Duijf PHG, Benezra R. The cancer biology of whole-chromosome instability. *Oncogene* 2013;32:4727–36.
- Heijink AM, Krajewska M, van Vugt MATM. The DNA damage response during mitosis. *Mutat Res* 2013;750:45–55.
- Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller M-C, Shaikh N, et al. Replication stress links structural and numerical cancer chromosomal instability. *Nature* 2013;494:492–6.
- Thompson SL, Compton DA. Examining the link between chromosomal instability and aneuploidy in human cells. *J Cell Biol* 2008;180:665–72.
- Janssen A, van der Burg M, Szuhai K, Kops GJPL, Medema RH. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science* 2011;333:1895–8.
- Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, et al. DNA breaks and chromosome pulverization from errors in mitosis. *Nature* 2012;482:53–8.
- Bakhoun SF, Kabeche L, Murnane JP, Zaki BI, Compton DA. DNA-damage response during mitosis induces whole-chromosome missegregation. *Cancer Discov* 2014;4:1281–9.
- Stolz A, Ertych N, Kienitz A, Vogel C, Schneider V, Fritz B, et al. The CHK2-BRCA1 tumour suppressor pathway ensures chromosomal stability in human somatic cells. *Nat Cell Biol* 2010;12:492–9.
- Takada S, Kelkar A, Theurkauf WE. Drosophila checkpoint kinase 2 couples centrosome function and spindle assembly to genomic integrity. *Cell* 2003;113:87–99.
- Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. *N Engl J Med* 2005;352:476–87.