IN THE SPOTLIGHT

Tuning Chromosomal Instability to Optimize Tumor Fitness

Mark E. Burkard[1,2] and Beth A. Weaver[2,3]

Summary: Low rates of chromosomal instability (CIN) are weakly tumor promoting, whereas high rates of CIN cause cell death and tumor suppression. In this context, Sansregret and colleagues show that one mechanism to restrain excessive CIN in tumor cells and increase fitness is through mutations in the anaphase promoting complex/cyclosome. This serves to delay mitotic progression and decrease the rate of chromosome missegregation.
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See related article by Sansregret et al., p. 218 (5).

Chromosomal instability (CIN), the recurrent gain and loss of chromosomes over multiple cell divisions, is common in cancer. Genetic diversity due to CIN provides a basis for clonal evolution and selection and is associated with higher tumor grade and reduced survival. Although modest levels of genetic diversity provide an advantage in tumors, excessively high rates of CIN cause tumor death, as inviable progeny emerge from each cell division (1). As a result, cancers often have low rates of CIN (2). Numerical CIN occurs primarily in mitosis, a time when entire chromosomes are in jeopardy of being missegregated into the incorrect daughter cells. Although low rates of CIN can promote cancer development in animal models and in humans, CIN is not always tumor promoting. Some animal models exhibiting low CIN have no increase in spontaneous or induced cancer incidence, whereas high rates of CIN increase cell death and suppress tumors, likely due to the lethal effect of loss of both copies of one or more essential chromosomes (reviewed in ref. 3). These considerations suggest that optimal fitness for cancer cell survival is achieved by adjusting CIN into an appropriate range. Yet how cancers might tune CIN has remained obscure.

The mitotic checkpoint (also known as the spindle assembly checkpoint) is the major cellular regulatory mechanism acting during mitosis to prevent CIN. Previous evidence from cell culture and animal models lacking numerous CIN genes has shown that the mitotic checkpoint is essential for viability (3). However, Wild and colleagues (4) recently found that cancer cells can survive homozygous deletion of the mitotic checkpoint gene *MAD2*, which is generally lethal, if they have substantially impaired activity of the anaphase promoting complex/cyclosome (APC/C). The APC/C is the E3 ubiquitin ligase that targets mitotic proteins for subsequent degradation and governs commitment to chromosome segregation in anaphase. If commitment to anaphase occurs before all chromosomes have made stable attachments to spindle microtubules via their kinetochores, chromosomes missegregate, resulting in CIN. To limit this, the mitotic checkpoint produces a diffusible signal that restrains APC/C activity in the presence of one or more unattached kinetochores. Thus, complete inactivation of the mitotic checkpoint produces high CIN and cell death, unless a decline in APC/C activity extends mitosis sufficiently to permit stable attachment of chromosomes for accurate segregation (Fig. 1).

In this issue, Sansregret and colleagues (5) screen for cellular mechanisms to survive CIN due to partial inhibition of MPS1 (also known as TTK), a kinase necessary to sustain the mitotic checkpoint signal. The rate of CIN is directly proportional to the dose of the MPS1 inhibitor. From a basal level of 1 chromosome missegregation event per 165 divisions in immortalized nontransformed human cells, increasing the MPS1 inhibitor causes a gradual increase in CIN from a low rate (1 missegregation every 2 divisions) to a high rate (5.3 chromosomes missegregated per division). Although reduction of the p53 tumor suppressor allowed increased cellular proliferation in response to low CIN, this tolerance reached a ceiling. High CIN severely impaired proliferation in the presence and absence of p53, demonstrating that CIN must be maintained below this threshold to maintain viability. To identify additional modifiers of the ability to tolerate CIN, an RNAi synthetic viability screen was performed to identify genes whose reduction promoted survival after MPS1 inhibition. Seven APC/C subunits and one of its E2 ligases were identified. Similar to the findings of Wild and colleagues, depletion of APC/C subunits or inhibition of APC/C extended mitotic duration, permitting accurate chromosome congression with reduced CIN in the presence of the MPS1 inhibitor. To determine whether this mechanism was recapitulated in cancer, the mutational status of APC/C components was examined. Across 30 cancer types, mutations in one or more of 15 APC/C subunits occurred in 0.7% to 22.9% of tumors. This does not exceed the expected mutation rate for a randomized group of 15 genes. However, the APC/C component *CDC27* was previously identified as a driver gene in colorectal cancer. Sansregret and colleagues found that truncation mutants of *CDC27* reduced APC/C function in

[1]Department of Medicine Hematology/Oncology, University of Wisconsin-Madison, Madison, Wisconsin. [2]UW Carbone Cancer Center, University of Wisconsin-Madison, Madison, Wisconsin. [3]Departments of Cell and Regenerative Biology and Oncology, University of Wisconsin-Madison, Madison, Wisconsin.

Corresponding Author: Beth A. Weaver, University of Wisconsin-Madison, 1111 Highland Avenue, 6109 WIMR, Madison, WI 53705-2275. Phone: 608-263-5309; Fax: 608-265-6905; E-mail: baweaver@wisc.edu

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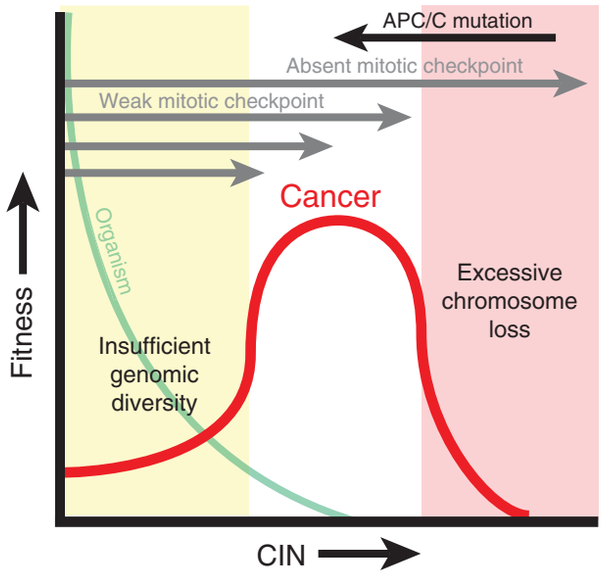

Figure 1. Tuning CIN to the optimal level. Fitness (vertical axis) is plotted against rate of CIN (horizontal axis). High levels of CIN cause excessive chromosome loss and cell death, whereas modest levels of CIN provide sufficient genetic diversity for cancer. The optimal range can be achieved by tuning CIN, which can be increased by weakening the mitotic checkpoint and decreased by reducing the activity of the APC/C.

fission yeast, and heterozygous mutation of *CDC27* delayed mitotic progression in human cells. Similarly, heterozygous disruption of *CDC27* in cancer cells delayed mitotic progression and decreased CIN. The reverse was also true—when cancer cells containing an endogenous mutation in the APC/C subunit *CDC23* had their APC/C function restored through CRISPR/Cas9-mediated genome editing, mitotic duration decreased and CIN increased. Taken together, these findings demonstrate that cancer cells can tune CIN to optimize fitness through manipulating APC/C activity.

One potential limitation is that known APC/C mutations are restricted to specific cancer types. However, most cancer genomic analyses have been performed in the primary tumor. Recent work has identified additional mutations that emerge with tumor progression and in response to selective pressures of treatment. It is therefore possible that additional mutations in APC/C components will be observed in advanced cancers, especially after prior treatments. Additionally, it is possible that larger-scale genomic aberrations such as translocations, amplifications, and deletions in APC/C components, or epigenetic, posttranscriptional, or posttranslational effects could modulate APC/C activity. Given the function of APC/C components in regulating CIN, a feedback loop is possible in that high CIN could restrain itself if, for example, a chromosome encoding an APC/C component is lost, thereby decreasing APC/C activity.

One promising avenue for cancer therapy is to increase CIN to lethal levels in tumors. Because many cancers have underlying CIN, they are expected to be more susceptible than normal dividing cells to drugs that elevate CIN. In this manner, these drugs are predicted to spare proliferating tissues that lack CIN (6). Although a number of therapeutics take this approach, inhibitors of MPS1 are expected to be most specific for elevating CIN without causing mitotic arrest. Two MPS1 inhibitors currently in development are CFI-402257 and BAY1161909 (clinicaltrials.gov numbers NCT02792465 and NCT02138812). The new findings from Sansregret and colleagues and Wild and colleagues suggest a mechanism of resistance via mutations in APC/C components. Additionally, primary resistance is anticipated in tumors that harbor mutations in APC/C components, which is seen in up to 22.9% of some tumor types. As discussed by Sansregret and colleagues, these agents may be most useful in tumor types that do not harbor *CDC27* mutations. Nevertheless, if mutations in APC/C components emerge in tumors treated with MPS1 inhibitors, this would provide strong genetic evidence of on-target effects of these drugs and confirm that that their cytotoxicity results from elevating CIN. However, there may be nongenetic routes to lengthening mitosis prior to anaphase onset. For example, cells adapted rapidly in culture to MPS1 inhibition, and this adaptation did not involve mutation of APC/C components. If human tumors have this degree of nongenetic pliability, the drugs may remain ineffective.

A related question is whether APC/C mutations impart resistance to chemotherapy drugs such as taxanes. Emerging evidence suggests that taxanes operate by increasing CIN to lethal levels and that tumors with underlying CIN have enhanced sensitivity (7). Intriguingly, cancer types with the highest incidence of *CDC27* mutations—melanoma and renal cell cancer (http://cbioportal.org/)—do not respond to taxanes, bolstering this hypothesis. It will be important to directly test if specific genetic alterations that affect APC/C activity regulate sensitivity to clinically relevant doses of taxanes.

Beyond the cancer relevance, the new evidence provides important insight into the biological role of the mitotic checkpoint. The essential function of the mitotic checkpoint is merely to delay mitosis sufficiently to prevent high CIN. If mitotic delay can be provided through another mechanism, such as APC/C impairment, the mitotic checkpoint is no longer essential. This is consistent with previous evidence documenting species-specific differences in the essentiality of this checkpoint that correlate with the rate of CIN in the absence of mitotic checkpoint signaling.

The generality of APC/C tuning in cancer is not yet clear. Some cancers have other mechanisms of generating diversity without CIN, such as microsatellite instability. Moreover, other manipulations have been discovered that suppress CIN. For example, overexpression of BUBR1 and specific BUBR1 fragments can reinforce the mitotic checkpoint to enhance error correction (8). Similarly, increased activity of KIF2B and MCAK kinesins can suppress CIN in certain contexts (9). However, it remains to be seen whether these alterations occur naturally in cancer to prevent excessive CIN. Moreover, it is unclear whether these effects could be harnessed for a chemopreventive or therapeutic strategy. One recent study found that cancer cells are adept at "retuning" CIN to optimal levels (10), so pharmacologic manipulation of CIN may be a challenge. Nevertheless, Sansregret and colleagues have demonstrated that relatively minor delays in mitosis due to APC/C inhibition can substantially reduce CIN, and that APC/C mutations found in cancer reduce its activity. Thus,

tuning of APC/C activity is used by some cancers to restrain excessively high CIN by increasing the duration of mitosis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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