

Chromosomal Instability as a Driver of Tumor Heterogeneity and Evolution

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Large-scale, massively parallel sequencing of human cancer samples has revealed tremendous genetic heterogeneity within individual tumors. Indeed, tumors are composed of an admixture of diverse subpopulations—subclones—that vary in space and time. Here, we discuss a principal driver of clonal diversification in cancer known as chromosomal instability (CIN), which complements other modes of genetic diversification creating the multilayered genomic instability often seen in human cancer. Cancer cells have evolved to fine-tune chromosome missegregation rates to balance the acquisition of heterogeneity while preserving favorable genotypes, a dependence that can be exploited for a therapeutic benefit. We discuss how whole-genome doubling events accelerate clonal evolution in a subset of tumors by providing a viable path toward favorable near-triploid karyotypes and present evidence for CIN-induced clonal speciation that can overcome the dependence on truncal initiating events.

Cancer originates from a single cell that has acquired a number of central genetic lesions allowing it to rescind normal multicellular development and expand, in an uncontrolled fashion at the expense of the host (Hanahan and Weinberg 2011). Genetic alterations that allow the initial expansion are typically defined as truncal and have been the focus of much investigation, as they represent attractive therapeutic targets harbored by the entirety of the malignant population (Barber et al. 2015). These studies have shed light on the number of mutations necessary for tumorigenesis, the

order in which these mutations occur, and the specific types of alterations required to produce a malignant tumor (Bozic et al. 2010; Vogelstein et al. 2013; Tomasetti et al. 2014; Landau et al. 2015).

After acquiring truncal mutations, most tumors undergo a second phase of intratumoral diversification. Massively parallel sequencing of primary, metastatic, and relapsed tumors revealed frequent cancer subclones, which harbor genomic alterations that are not detected in the remainder of the tumor cell population. Notably, these subclones often show evidence sup-

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portive of positive selection in an active evolutionary process. In many tumors, subclonal mutations show marked enrichment of driver events, as well as convergent evolutionary processes whereby multiple subclones independently acquire mutations that activate similar oncogenic pathways (Gerlinger et al. 2012; Yates and Campbell 2012; Landau et al. 2013; 2015; Brastianos et al. 2015; Murugaesu et al. 2015; Stachler et al. 2015; Yates et al. 2015).

Fundamentally, subclonal heterogeneity stems from the ability of malignant cells to acquire novel mutations, a defining feature of many human tumors. Ongoing genomic diversification has been associated with poor prognosis and increased probability of tumor relapse after therapy (Landau et al. 2013, 2015; Andor et al. 2016; McGranahan et al. 2016). One of the central drivers of intratumoral diversification is copy number instability of particular loci, large chromosome segments, or entire chromosomes (Holland and Cleveland 2009; Navin et al. 2011; Wang et al. 2014). Indeed, cancer cells often show substantial diversity with respect to chromosomal content (karyotypes) (Gilgenkrantz and Rivera 2003). Aneuploidy, which represents a “state” of abnormal

karyotype, has long been recognized as a hallmark of tumorigenesis (Boveri 1903). Karyotypic heterogeneity in tumor cells draws its origin from “chromosomal instability” (otherwise known as CIN), a hallmark that distinguishes most cancers from its normal tissue counterpart. CIN does not only generate abnormal aneuploid karyotypes, but continually expands phenotypic heterogeneity as tumor cell populations undergo consecutive cell divisions (Fig. 1A) (Lengauer et al. 1998, 1997).

Aneuploidy is present in ~80% of human solid neoplasms (Ame et al. 2008) the majority of which often exhibit CIN (Storchova and Kuffer 2008). CIN can be present in two major forms: The first is numerical CIN whereby copy numbers of entire chromosomes vary within and among tumor cells. The second form is structural CIN, which is defined by copy number variations of subchromosomal regions (Roschke and Rozenblum 2014). Although both types of CIN arise through distinct cellular mechanisms and occur at different rates, they are nonetheless related and the presence of numerical CIN can induce, or accelerate, structural CIN and vice versa (Crasta et al. 2012; Bakhom et al. 2014a). For the purpose of this review, we will

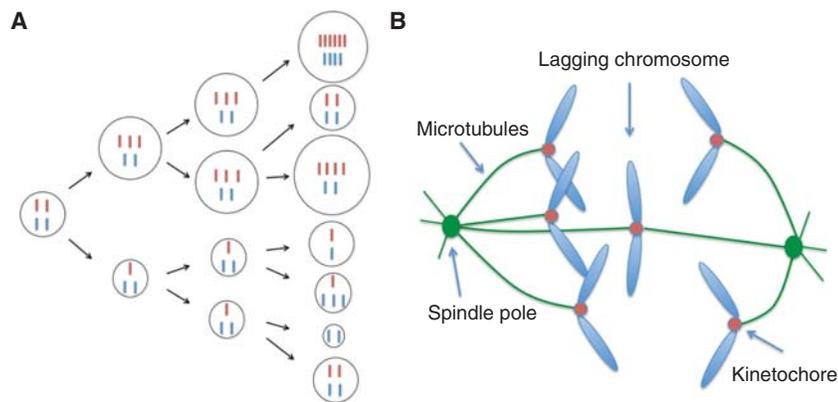


Figure 1. Chromosomal instability (CIN) in cancer. (A) Cancer cells experience frequent errors in the segregation of entire chromosomes, leading to karyotypic heterogeneity. Depending on the oncogenic and tumor suppressive effect of genes encoded by individual chromosomes, individual karyotypes will lead to distinct proliferative states and subclonal heterogeneity shaping the overall fitness of the population. Cell size depicts the relative cell fitness. Chromosomal instability (CIN) can enable rapid expansion of cells with variable fitness. (B) Lagging chromosomes during mitosis are a hallmark of CIN in cancer. They result from the erroneous attachments of chromosomes to spindle microtubules at the kinetochores. Most of the cellular defects that lead to CIN in cancer converge onto this process and produce lagging chromosomes.



focus primarily on the contribution of numerical CIN to intratumoral heterogeneity. However, we will also discuss how numerical and structural CIN are related and how the multilayered CIN phenotype can play different roles in tumor evolution under various selection pressures.

Numerical CIN is primarily defined by the “rate” at which chromosomes missegregate throughout consecutive cell divisions (Lengauer et al. 1998). The frequency of chromosome missegregation rates, directly measured in cancer-derived cell lines, are on the order of $\sim 1.3 \times 10^{-3}$ per chromosome copy per cell division, which is ~ 50 – 100 -fold higher than in normal nontransformed cells (Thompson and Compton 2008; Bakhom et al. 2009b; Laughney et al. 2015). This translates into a chromosome missegregation event every 3–10 cell divisions after accounting for cellular ploidy. In full-fledged malignancies, chromosome missegregation events may have a positive or negative effect on cellular fitness depending on the altered dosage of hundreds to thousands of genes carried by the missegregated chromosomes (Davoli et al. 2013; Laughney et al. 2015); making CIN a powerful tool to rapidly shape the phenotypic landscape of tumor cell populations.

THE ORIGINS OF CIN

A number of cellular mechanisms have been found to lead to CIN in cancer (reviewed in Thompson et al. 2010), including weakened sister chromatid cohesion (Zhang et al. 2008), deregulated centrosome duplication (Ganem et al. 2009; Silkworth et al. 2009), defects in microtubule attachments to chromosomes at the kinetochores (Cimini et al. 2001; Bakhom et al. 2009a,b), premitotic replication stress (Burrell et al. 2013), and mitotic DNA damage (Crasta et al. 2012; Bakhom et al. 2014a). Irrespective of the underlying cause, these mechanisms converge onto the process of chromosome segregation during mitosis, and lead to lagging chromosomes during anaphase, which is considered a hallmark of chromosomally unstable cells (Fig. 1B) (Cimini et al. 2001; Thompson and Compton 2011; Bakhom et al. 2014b). Chromosomes lag as a result of their simultaneous

attachments to microtubules emanating from opposite spindle poles (Cimini et al. 2003, 2002), which increases their potential for missegregation or encapsulation in micronuclei (Crasta et al. 2012).

The process of chromosome segregation is vulnerable in the face of many cellular defects that are known to occur in cancer. Activation of oncogenic pathways, loss of key tumor suppressor pathways, and a large number of therapeutic interventions can either facilitate or induce CIN (Orr and Compton 2013; Lee et al. 2016). This vulnerability stands in sharp contrast to the lack of aberrant karyotypes observed in normal human cells (Knouse et al. 2014). Although not yet fully understood, work in mice and cancer-derived cell lines revealed that the tumor suppressors p53 and p21 play a key role in safeguarding against aneuploidy. Mice without functional p53 generate tumors with aneuploid karyotypes (Shao et al. 2000). Furthermore, abrogation of p53 or p21 allows tolerance of aneuploidy and the propagation of instability (Thompson and Compton 2010). Accordingly, mutations in the p53 pathway are more prevalent in advanced tumors that typically contain significant chromosome copy number variations (Rivlin et al. 2011). More recently, the Hippo tumor suppressor signaling pathway was shown to be activated in response to cytokinesis failure, thereby acting as a selective safeguard against tetraploidy but not aneuploidy (Ganem et al. 2014).

THE CONSEQUENCES OF ANEUPLOIDY

Even in a potentially permissible cellular environment lacking p53, there is strong evidence to show that induction of aneuploidy in diploid cells is associated with reduced fitness and delays tumor formation (Rowald et al. 2016; Sheltzer et al. 2016). Work in yeast and human cells shows that aneuploidy induces metabolic and proteotoxic stress, which translate into lower proliferation rates (Torres et al. 2007, 2010; Williams et al. 2008; Sheltzer and Amon 2011; Oromendia et al. 2012; Sheltzer et al. 2012; Sheltzer 2013; Dürrbaum et al. 2014). Given the increased metabolic demand and oxidative stress imparted by aneuploidy, this has led some to postulate that

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aneuploidy may serve as one of the triggers for the Warburg effect often seen in cancer, whereby cells shift away from oxidative phosphorylation toward aerobic glycolysis (Vander Heiden et al. 2009; Siegel and Amon 2012). Inducing aneuploidy in vivo through depletion of Cenp-E—a mitotic centromere protein involved in chromosome segregation—reduces the incidence of hepatocellular carcinomas (Weaver et al. 2007) and computational evidence suggests that the percent reduction in normal cell fitness in response to aneuploidy could range anywhere from 6% to 30% (Gusev et al. 2001; Valind et al. 2013).

Although aneuploidy leads to a global reduction in cellular fitness, it also enhances the ability of malignant populations to sample the fitness landscape for superior evolutionary trajectories. There are many examples in which the induction of aneuploidy and CIN leads to tumorigenesis. For instance, induction of CIN through overexpression of *mad2*, a spindle assembly checkpoint protein, leads to spontaneous tumor formation (Schvartzman et al. 2011). Likewise, depletion of Cenp-E leads to the formation of lymphomas and lung tumors, and overexpression of *hec1*, a kinetochore protein involved in chromosome segregation, also leads to spontaneous tumor and adenoma formation (Díaz-Rodríguez et al. 2008). It is important to note, however, that in many of these cases, secondary alterations were required for tumorigenesis, such as the deletion of *p53*, *p21*, or *p19^{Arf}* (Fujiwara et al. 2005; Weaver et al. 2007). Thus, aneuploidy can have both oncogenic and tumor suppressive phenotypes in a context-dependent manner. The determinants of this dichotomous phenotype are still poorly understood; some have postulated that the degree of aneuploidy is an important determinant of its effect on tumorigenesis (Silk et al. 2013); however, this concept requires additional experimental validation.

WHOLE-GENOME DOUBLING DURING TUMOR EVOLUTION

Chromosomally unstable clones have a wide array of karyotypes available for them to sample. For example, a cell containing up to six copies of

any given chromosome has 6^{23} unique karyotypic combinations. Our understanding of how CIN enables tumor subclones to sample this vast aneuploid fitness landscape is only beginning to emerge. Laughney et al. (2015) explored this by allowing clonal populations to sample the aneuploid fitness landscape in silico, and found that they strikingly converged onto a favorable near-triploid state that maximized the numbers of chromosomes that are more likely to harbor oncogenes and minimize the copies of chromosomes that are more likely to harbor tumor suppressor genes (Fig. 2). Importantly, both diploid-derived and tetraploid-derived clones achieved this optimal karyotype state, yet at a much lower fitness cost for the latter. This was due to the ability of tetraploid-derived clones to buffer chromosome loss and avoid lethal nullisomy. This suggests that a tetraploid intermediate arising from whole genome doubling is a desirable path toward the commonly observed favorable near-triploid state.

Consistent with this framework, copy number analysis across cancer revealed a propensity

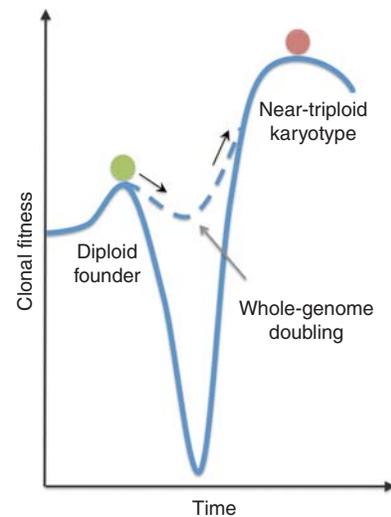


Figure 2. Experimental and computational work reveals a favorable near-triploid karyotype, which favors clonal fitness. Diploid-derived and tetraploid-derived populations alike can achieve this karyotype, albeit at a much lower fitness cost for the latter, suggesting that whole-genome doubling provides a more cost-effective path toward a highly favorable state.

for a near-triploid state (Storchova and Kuffer 2008; Laughney et al. 2015). Interestingly, Storchova and Kuffer found that intratumor karyotypic heterogeneity was maximal in tumors with near-triploid karyotypes, which may signify that higher rates of CIN allow a more rapid convergence onto this favorable state, or that the near-triploid state allows more facile clonal diversification (Storchova and Kuffer 2008). Second, genomic analysis also supports the presence of a tetraploid intermediate occurring early during tumor evolution followed by gradual chromosome loss in at least a third of tumor samples examined (Carter et al. 2012; Zack et al. 2013). Accordingly, experimental work in yeast, cancer cell lines, and xenograft mouse models shows that inducing whole-genome doubling promotes tumorigenesis and propagates further genomic instability (Fujiwara et al. 2005; Dewhurst et al. 2014; Selmecki et al. 2015).

Given the facilitating impact of genome duplication on cancer, it is not surprising that many factors safeguard against the proliferation of tetraploid cells either before transformation or in the early stages of tumorigenesis (Ganem and Pellman 2007). Just like aneuploidy induction, polyploidization induces a p53-mediated cell-cycle arrest (Carter 1967; Fujiwara et al. 2005). This arrest is likely instigated by DNA damage and other osmotic and proteotoxic stressors that are mediated by p38/MAP kinase (Castedo et al. 2006; Srsen et al. 2006). More recently, Ganem et al. (2014) identified the Hippo tumor suppressor pathway as a tetraploid-specific checkpoint that responds to cytokinesis failure. Activation of this pathway is mediated in part by the presence of extra centrosomes that alter small G-protein signaling, which activate LATS2 kinase. LATS2 in turn inhibits the transcriptional regulators YAP and TAZ through a p53-dependent mechanism. Interestingly, this pathway was selectively activated in response to whole-genome doubling and not in response to whole-chromosome missegregation, highlighting the specificity of this pathway toward tetraploidy.

Thus, whole-genome doubling is a key step in the “evolution” of the multicellular genome

to the unicellular-like cancer genome. Consistently, powerful mechanisms have evolved to prevent whole-genome doubling (Ganem and Pellman 2007; Mable 2013); however, once cells are able to overcome these safeguards whole-genome doubling generates a more plastic genome that empowers the evolutionary process and confers higher adaptive potential to the malignant population. The exact contribution of whole-genome doubling during early and late tumor evolution remains to be established. Although tetraploidization may make it harder for cells to fully inactivate key tumor suppressor genes, it is likely that the ratio of oncogenes-to-tumor suppressor genes is the major determinant of tumor fitness. Moreover, the presence of multiple copies of each chromosome allows the cells to shuffle their chromosome numbers with a lower risk of lethal nullisomy, where all copies of an essential gene are lost. In summary, the recurrent patterns of chromosome copy number changes seen across human cancer, as well as the observation of convergent evolution toward near triploid karyotypes, show that chromosome missegregation is not a mere bystander during tumor evolution, but actively contributes to malignant speciation.

LINKING NUMERICAL CIN TO STRUCTURAL CIN AND CHROMOTHRIPSIS

Numerical CIN does not exist in isolation. Numerical chromosomal aberrations are often coinherited with complex structural damage that alters the juxtaposition of genomic loci (e.g., translocations, inversions, duplications, etc.). Although they occur through distinct mechanisms and at different rates, structural and numerical CIN are interrelated (Sheltzer et al. 2011; Crasta et al. 2012; Burrell et al. 2013; Bakhoum et al. 2014a). Replication stress and structural chromosomal damage can lead to defective mitotic chromosome segregation as both acentric chromosomes and dicentric chromosomes cannot form proper bi-oriented attachments to the mitotic spindle (Murnane 2012; Burrell et al. 2013). Additionally, activation of the DNA damage response during mitosis, either through extrinsic fac-

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tors or uncorrected premitotic defects, perturbs microtubule attachments to chromosomes and leads to whole-chromosome segregation errors and CIN (Bakhoun et al. 2014a; Orthwein et al. 2014).

Conversely, chromosome missegregation during mitosis can also lead to abrupt structural chromosomal aberrations. Lagging chromosomes are repeatedly encapsulated in micronuclei, which are defective in DNA replication and repair and have dysfunctional micronuclear envelopes (Crasta et al. 2012). Such micronuclei undergo catastrophic micronuclear envelope collapse associated with invasion of the endoplasmic reticulum tubules into their enclosed chromatin (Hatch et al. 2013). Through a yet unidentified mechanism, exposure of this double-stranded DNA to the cytosol leads to dramatic chromosome pulverization (Fig. 3) (Crasta et al. 2012). By performing single-cell sequencing, Zhang et al. (2015) were able to show that pulverized chromosomes can be repaired through nonhomologous end joining, leading to massive chromosome rearrangements, a process known as “chromothripsis” (Stephens et al. 2011). This mitotic pathway of-

fers the means to dramatically alter the genome in the span of a single cell division. Chromothripsis can also occur as a result of erroneous segregation of dicentric chromosomes resulting from telomere fusion (Maciejowski et al. 2015). It is likely that most chromothripsis events lead to unviability—as cells lose chromosome fragments containing essential genes (Zhang et al. 2015)—although rarely chromothripsis leads to amplification or fusion events that significantly enhance cellular fitness. Indeed, in their original report of chromothripsis, Stephens et al. (2011) found that this process is associated with the formation of oncogene-harboring double-minute chromosomes that existed in hundreds of copies per cell.

The rapid, punctuated genomic changes that result from such genomic “catastrophes,” stands in stark contrast to the progressive genomic alteration caused by numerical changes in chromosome copy numbers. The interconnectedness between structural and numerical CIN offers tumor cell populations the ability to alternate between these two rates of genomic transformation during the process of cancer evolution.

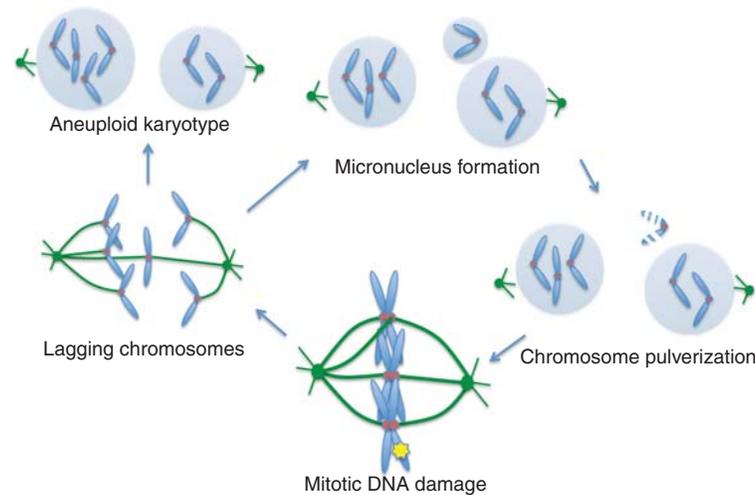


Figure 3. Diagram depicting the link between numerical and structural chromosomal instability (CIN). In addition to aneuploid karyotypes, chromosome segregation during mitosis also leads to the formation of chromosome-containing micronuclei. This in turn leads to pulverization of their enclosed chromosomes and persistence of DNA damage into the subsequent mitosis. Activation of a partial mitotic DNA damage response in turn leads to whole chromosome segregation forming a cycle linking numerical and structural CIN.

THE DYNAMICS OF CIN IN TUMOR CLONAL EVOLUTION AND THERAPEUTIC RESPONSE

How distinct chromosome copy numbers alter tumor cell fitness remains a poorly understood process. Chromosome (or arm)-level amplifications and deletions alter transcript and protein levels of hundreds to thousands of genes (Torres et al. 2007; Williams et al. 2008) and have often been associated with altered prognosis and different tumor behavior.

The identification of genes on these chromosome segments responsible for altered prognosis has been challenging. A recent attempt by Roy et al. (2016) used a multitiered genomic dissection strategy and patient data to identify a handful of genes responsible for poor prognosis in low-grade gliomas harboring loss of the 9p chromosome arm. The investigators found that reductions in messenger RNA levels in response to 9p loss was due to haploinsufficiency in subset of genetic loci. In another subset, there was evidence for epigenetic inactivation on the remaining copies of the 9p chromosome arms leading to significant down-regulation of transcript levels. Interestingly, many genes on this chromosome arm were not significantly affected suggesting a dosage compensation mechanism.

In a separate study, Davoli et al. (2013) mapped tumor suppressor genes and oncogenes on human chromosomes inferred from the mutational patterns of these genes in human tumors. By analyzing the potency and density of oncogenes and tumor suppressor genes, they found that human chromosomes are not equivalent with respect to the net cumulative oncogenic and tumor suppressive effect of the genes they encode, suggesting that cumulative haploinsufficiency and triplosensitivity of pro- and antiproliferative genes shape tumor karyotypes and in part explains chromosome-level amplifications and deletions in cancer. This work provides a framework to explain how chromosome copy number changes that affect hundreds of genes may still output a coherent fitness effect, and therefore subjected to evolutionary selection.

To quantitatively model the evolutionary impact of CIN, Laughney et al. (2015) simulated the process of clonal evolution under chromosomally unstable conditions. Cell viability was determined by the net dosage of pro- and antiproliferative genes they contained, dictated by the respective chromosome copy numbers (Fig. 1A). Interestingly, the dependence of clonal fitness on chromosome missegregation rates was nonlinear and parabolic—fitness rapidly declined when missegregation rates were either too high or too low. At very low rates, populations lacked the ability to generate phenotypic heterogeneity induced by karyotypic diversity. As such, clonal populations were less capable of sampling fitter karyotypic states. On the other hand, excessively high rates of chromosome missegregation prevented populations from preserving highly fit karyotypic states, and also resulted in frequent nullisomy—the lethal loss of all copies of any given chromosome. Thus, tumor populations thrive when they balance the ability to diversify with the ability to preserve favorable genotypes. Strikingly, when missegregation frequencies were experimentally measured in cancer-derived cell lines they largely clustered around the range predicted to be optimal for population fitness ($\sim 1.9 \times 10^{-3}$ chromosome per copy per cell division), suggesting a strong evolutionary pressure to optimize CIN rates in human cancer (Fig. 4) (Laughney et al. 2015).

The concept of an optimal range of chromosome missegregation rates has important therapeutic implications (Silk et al. 2013; Laughney et al. 2015). It predicts that tumors with moderate CIN (within the optimal range) would show the highest likelihood of stable plasticity promoting therapeutic resistance. On the other hand, tumors would be vulnerable to either a decrease or an increase of CIN rates outside of the optimal range that supports clonal evolution. This is indeed supported by a number of clinical observations and experimental evidence (Bakhoum et al. 2011; Birkbak et al. 2011; Roylance et al. 2011; Jamal-Hanjani et al. 2015; Andor et al. 2016). Among colorectal cancer-derived cell lines, chromosomally unstable cells are more resistant to targeted kinase inhibition

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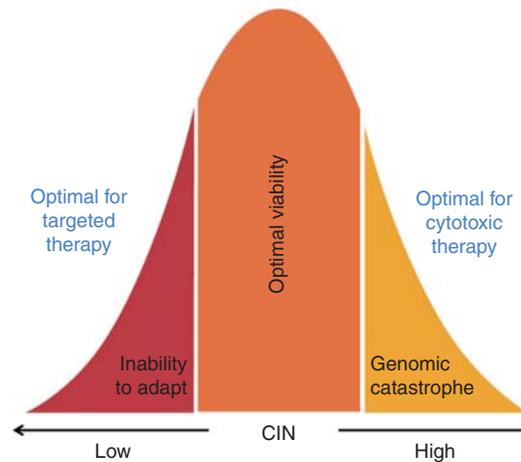


Figure 4. Relative tumor clonal fitness as a function of chromosomal instability (CIN). Excessively low CIN would suppress genomic heterogeneity leading to reduction in tumor adaptability, whereas excessively high CIN would lead to genomic collapse, DNA damage, and frequent nullisomy events incompatible with viability.



as compared with their chromosomally stable counterparts (Lee et al. 2011). In patients with breast cancer, the presence of moderate CIN is associated with inferior prognosis and resistance to taxanes whereas extreme CIN is associated with improved survival and reduced tumor relapse after primary therapy (Swanton et al. 2009; Roylance et al. 2011; Jamal-Hanjani et al. 2015). Furthermore, in a pancancer genomic analysis of intratumor heterogeneity, patients that had tumors in the middle quartiles of chromosome copy number variations were at a significantly greater risk for death compared with those with tumors in the lowest or highest quartiles of chromosome copy number variations (Andor et al. 2016).

This notion offers a largely untapped opportunity to target chromosomally unstable tumors, by increasing CIN beyond the tolerable threshold. Support for this strategy stems from observations that CIN is a predictor of favorable response to therapies that induce chromosome missegregation, particularly conventional DNA-damaging cytotoxic therapies. For instance, CIN correlates with sensitivity to platinum-based therapies in ovarian cancer (Swanton et al. 2009), and in those with locally advanced rectal cancer, considerably elevated rates of chro-

mosome missegregation are associated with a higher likelihood of pathologic response after treatment with radiation therapy and concurrent 5-fluorouracil (Zaki et al. 2014). Consistent with this notion, in the pancancer analysis performed by Andor et al. (2016), the negative impact of CIN on survival was abolished in the subset of patients who received DNA-damaging therapies (Fig. 4).

Thus, elevating chromosome missegregation rates in inherently chromosomally unstable cancers has been proposed as a potential anti-neoplastic strategy. Experimental evidence shows that cells are rendered more sensitive to agents that increase chromosome missegregation when they already experience preexisting CIN (Janssen et al. 2009; Bakhoun et al. 2015). The recent development of specific inhibitors that are capable of inducing massive chromosome missegregation is encouraging and has shown substantial preclinical promise (Mason et al. 2014). These agents can be rationally combined with traditional therapies, such as DNA-damaging agents, to achieve greater therapeutic benefits. Collectively, a better understanding of the pretreatment CIN status as well as the reciprocal effect of therapy on chromosome stability will enable us to design strat-

egies that aim at displacing cells from their acquired favorable copy number states.

CIN AND THE EMERGENCE OF SUBCLONAL THERAPEUTIC RESISTANCE

The ability for CIN to enable phenotypic diversity among tumor cells can accelerate the emergence of subclones with specific genotypes that confer therapeutic resistance. For instance, treatment with EGFR inhibitors rapidly selects for glioblastoma-derived cells with amplification of the number of copies of chromosome 7, which harbors the *EGFR* gene (Chen et al. 2015).

In addition to directly altering the copy number of the target gene, CIN has the potential to induce resistance by circumventing driver alterations and relieving tumors from their dependence on oncogenic events that underlie their genesis. This was experimentally tested by Sotillo et al. (2010) where they induced CIN in Kras-driven lung tumors through transient overexpression of Mad2. Subsequent withdrawal of Kras led to tumor regression; however, transient Mad2 overexpression promoted tumor relapse after Kras withdrawal. These Kras-independent tumors were characterized by markedly elevated rates of aneuploidy and CIN. In these tumors, the investigators found up-regulation of proliferative pathways as evidenced by elevated levels of phosphorylated forms of ERK, Stat3, and AKT. Other studies revealed that CIN can circumvent the loss-of-function of otherwise essential genes in yeast (Rancati et al. 2008; Liu et al. 2015). Collectively, these findings support the notion that CIN represents an adaptive cellular tool to overcome inhibition of essential genes or truncal genetic alterations that occur early during tumor evolution.

CONCLUDING REMARKS

Understanding the mechanisms and dynamics of tumor genomic diversification is critical to our ability to overcome the challenge of drug resistance. To target CIN therapeutically, we require a deeper understanding of several key as-

pects of CIN in cancer. These include identifying more comprehensively the cellular mechanisms that underlie CIN and enable tolerance for a chromosomally unstable state. Second, because CIN is defined by a rate of chromosome mis-segregation, methods interrogating this process in cancer should be refined to not only measure the extent of chromosome-level copy number alterations in cancer but also the rate at which chromosome number changes throughout consecutive cell divisions. Widely used methods such as comparative genomic hybridization and DNA sequencing of bulk tumor samples significantly mask the extent of karyotypic cell-to-cell variation, which may now be extensively probed through single-cell analyses (Laughney et al. 2015; Bakker et al. 2016). Third, our efforts to understand chromosome copy number changes would need to be coupled with quantitative models that aim to predict the behavior of tumor cell populations derived from our knowledge of chromosome segregation at the single-cell level. Ultimately, these models hold the promise of providing robust predictive tools enabling precision therapeutic manipulation of the rapidly evolving cancer genome.

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