Trends in Cancer

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Opinion

Difference Makers: Chromosomal Instability versus Aneuploidy in Cancer

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Human cancers harbor great numbers of genomic alterations. One of the most common alterations is aneuploidy, an imbalance at the chromosome level. Some aneuploid cancer cell populations show varying chromosome copy number alterations over time, a phenotype known as 'chromosomal instability' (CIN). Chromosome segregation errors in mitosis are the most common cause for CIN *in vitro*, and these are also thought to underlie the aneuploidies seen in clinical cancer samples. However, CIN and aneuploidy are different traits and they are likely to have distinct impacts on tumor evolution and clinical tumor behavior. In this opinion article, we discuss these differences and describe scenarios in which distinguishing them can be clinically relevant.

Aneuploidy and Chromosomal Instability in Cancer

The development of neoplastic lesions is accompanied by the accumulation of genomic mutations [1]. Recent sequencing efforts greatly enhanced our understanding of cancer genomes and their evolution. These studies strongly suggest that elevated mutation rates combined with evolutionary dynamics ultimately ensure clonal expansion of tumor cells, giving rise to heterogeneous tumors [2,3].

One of the most dramatic manifestations of genomic alterations in cancer is aneuploidy. Aneuploidy describes a karyotype that deviates from an exact multiple of the haploid set of chromosomes, resulting in genetic imbalances. The presence of aneuploidy in cancer is widespread and diverse [4], with great heterogeneity within tumors [5–7] and between different tumor types. For instance, about 65% of lung tumors [8] and 91% of glioblastomas [9] are reported to be aneuploid, whereas aneuploidy is rare in early stage prostate cancer [10,11]. A recent study estimated that approximately 70% of all human tumors are aneuploid [9]. In addition, on average, approximately 30% of the genome of a tumor shows copy number alterations [12].

Cancer genomes are generally dynamic, owing to underlying genetic instability phenotypes and changes in selective pressures in the course of tumorigenesis [1,13]. Different mechanisms of genomic instability have been described and each can be recognized by specific mutational signatures [13,14]. Instability is also observed at the whole chromosome level and was first described to be present in cell lines derived from colorectal tumors [15]. By determining the copy number of specific chromosomes in single cells, it was shown that the chromosomal content within certain cell populations varied over time. This phenotype was named 'chromosomal instability' (CIN), and was proposed to result from errors in chromosome segregation during mitosis. Indeed, CIN cell lines typically show decreased mitotic fidelity [16–18]. CIN can have additional causes, including erroneous or unfinished replication [19] and entosis [20]. For simplicity's sake, we refer to cells that evolve novel karyotypes over time as CIN, irrespective

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The majority of human tumors are aneuploid and tumor cell populations undergo karyotype changes over time *in vitro*. An elevated rate of chromosome segregation errors [known as 'chromosomal instability (CIN)'] is believed to underlie this.

Besides ongoing mitotic errors, evolutionary dynamics also impact on the karyotypic divergence in tumors. CIN can therefore not be inferred from karyotype measurement (e.g., in aneuploid tumors).

CIN, aneuploidy, and karyotype divergence are three fundamentally different traits, but are often equated in literature, for example, to describe aneuploid tumors.

Recent advances suggest that discriminating CIN and aneuploidy might be clinically relevant.

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of underlying mechanisms, but note that it most commonly relates to errors during mitotic chromosome segregation.

Confusingly, the term CIN has been widely used not only to describe the tendency for mitotic errors in cells that lead to karyotype divergence, but also in the classification of aneuploid tumors to distinguish them from hypermutated tumors. As such, 'CIN' is a term used to describe fundamentally different traits. Recent advances, however, have led to plausible hypotheses on how aneuploidy and CIN may differentially affect tumor behavior, therapy response, and tumor relapse. We therefore argue that considering them as distinct traits is relevant for cancer research and therapy. In this opinion article, we highlight data that underscore the differences between aneuploidy and CIN and provide scenarios in which the distinction can be clinically relevant.

Chromosomal Traits: Definitions, Differences, and Interplay

Aneuploidy, karyotype divergence, and CIN are thus closely linked but not identical traits (our operational definitions of the three chromosomal traits – aneuploidy, CIN, and karyotype divergence – are clarified in Box 1). In principle, karyotype divergence can result solely from evolutionary dynamics in a heterogeneously aneuploid population, and therefore, although CIN at one time caused the heterogeneity, CIN is not necessarily present in such populations (Figure 1A). The evolutionary dynamics are likely driven by selection for optimal karyotypes, but might also result from genetic drift (Figure 1A, Population 3 to 4 and Population 2 to 3, respectively). An example of such dynamics is the big bang model of colorectal cancer evolution, in which catastrophic events (possibly mitotic errors) in a primordial tumor cell generate a population of cells with a high level of genetic variation. This population then expands to generate intermixed subclones, which change over time as a result of evolutionary dynamics [21]. Interestingly, this model has recently been proposed to explain punctuated karyotype evolution in triple-negative breast tumors [22]. Karyotype divergence can thus be present in the absence of CIN. One important implication from this notion is that the level of CIN cannot be inferred from karyotype measurements. This concept is further clarified in Figure 1A, which shows the progression of a cell population from homogeneously diploid (Figure 1A, Population 1) to heterogeneously aneuploid (Populations 2, 3, and 4) but with distinct causes for karyotype changes at different stages in the life of the population. Importantly, karyotype measurements from the Populations 2, 3, and 4 will show karyotype divergence, and CIN may falsely be inferred.

Several observations support the notion that CIN and aneuploidy are not necessarily directly correlated. A case in point is the human colorectal carcinoma cell line HCT 116 that is aneuploid but mitotically stable. In addition, single-cell sequencing (SCSing) of breast cancers suggested that in those patients, aneuploid rearrangements occurred early but were then maintained stably since there were little karyotypic variances between cells [22–24]. The breast cancer cells may

Box 1. Operational Definitions of Aneuploidy, Karyotype Divergence, and Chromosomal Instability (CIN)

In order to clearly discriminate between the chromosomal traits, we here provide our operational definitions as used throughout this opinion article:

- Aneuploidy: the state of having a karyotype that deviates from an exact multiple of the haploid set of chromosomes, and thus, refers to the 'genotype'. The level of aneuploidy refers to the number of imbalanced chromosomes.
- Karyotype divergence: a 'population phenotype' in which fractions of cells with specific karyotypes change over time. The level of divergence refers to the changes in size and number of fractions over time.
- CIN: the acquired elevated chance of unequal distribution of genomic content over two daughter cells, and is thus a 'cellular phenotype'. The level of CIN refers to the frequency and severity of these errors over successive cell-division cycles.

Although the three traits are intimately linked, they are fundamentally different. Therefore, the presence of one does not necessarily require the presence of another at a given point in time. For instance, CIN leads to aneuploid progeny and karyotypically divergent populations, but an aneuploid cell or cells within divergent populations do not necessarily remain CIN (Figure 1A). The CIN phenotype can be temporary (e.g., due to environmental factors) and the resulting aneuploid progeny may regain the ability for high-fidelity mitotic cell divisions.

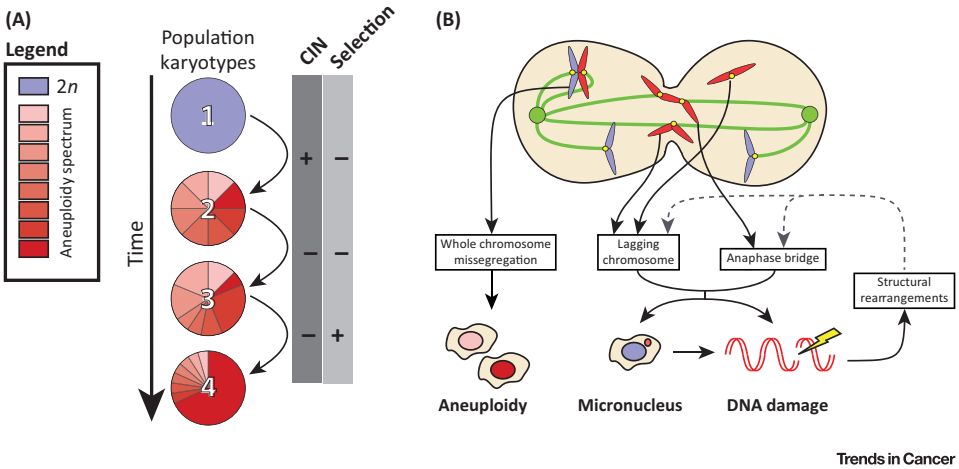

Figure 1. Distinguishing Aneuploidy, Karyotype Divergence, and Chromosomal Instability (CIN). (A) During the generation of a heterogeneously aneuploid population (e.g., a tumor), the karyotypes in the population are initially caused by CIN and subsequently selected for via evolutionary mechanisms. Once a heterogeneous population has been formed, CIN is no longer essential to cause karyotype divergence. The level of aneuploidy or karyotype divergence, therefore, does not necessarily reflect the level of CIN. Pie charts depicting cell populations; the colors reflect diploid (blue) or different aneuploid (shades of red) karyotypes. (B) Chromosome missegregation can occur in different forms. Whole chromosome missegregation leads to aneuploid progeny. Lagging chromosomes and anaphase bridges can cause the formation of micronuclei and DNA damage. Erroneous repair can subsequently result in chromosome rearrangements that in turn can give rise to lagging chromosomes and anaphase bridges.

thus not be CIN. Finally, a recent study showed that nonregenerating tissues in a CIN mouse strain are highly aneuploid while regenerating tissues were not [25]. Aneuploidy measurements thus do not directly relate to the level of mitotic errors in these mice.

In conclusion, aneuploidy, karyotype divergence, and CIN are linked but distinct traits. Importantly, since unstable aneuploidies in a population can result from ongoing mitotic errors and also from evolutionary dynamics, a CIN phenotype cannot be directly inferred from static karyotype measurements in experimental or clinical samples.

CIN versus Aneuploidy on Cellular Fitness and Proliferation

Although debated for the liver and brain [26–32], aneuploidy is rare in adult tissues. This is in agreement with the incompatibility of constitutional aneuploidies with organismal development: in humans, all autosomal aneuploidies, with the exception of trisomies 13, 18, and 21, are embryonic lethal [33]. By contrast, some patients with somatic mosaic and variegated aneuploidies can survive until well into childhood, but suffer from mental and growth retardation and delayed development [34].

In accordance with the problems that present at the organismal level, aneuploidy decreases fitness at the cellular level. Both mammalian and yeast cells have reduced proliferation rates when aneuploid [35–38], mainly resulting from general aneuploidy-induced proteotoxic and metabolic stresses [35,39,40]. The association of aneuploidy with decreased cellular fitness on the one hand and cancer on the other appears counterintuitive. In fact, cancer cells are the only cell type known to maintain or even increase their proliferation rate while being aneuploid. This ‘aneuploidy paradox’ in cancer has raised questions regarding the causative roles of mitotic errors and aneuploidy in oncogenesis [37]. The aneuploidy paradox is not yet resolved, but it seems likely that cancer cells have acquired aneuploidy tolerance, either by mutations or by altered environments that favor the proliferation of aneuploid cells. The acquisition of aneuploidy tolerance was shown to be a plausible scenario in yeast [41,42].

Mitotic errors lead to aneuploid progeny and therefore impaired fitness. In addition, chromosome missegregation events can induce DNA damage and thereby activate DNA-damage response mechanisms that generally lead to p53-mediated cell-cycle arrest or apoptosis [43–46]. The nature of the CIN phenotype, however, potentially enables acquisition of compensatory mutations that allow cell-cycle progression of aneuploid cells. For example, disruption of p53 function is a widely used approach to ensure cell-cycle progression of cells after a mitotic error. Therefore, if the error itself affects the p53 locus, cells may be able to escape cell-cycle arrest or apoptosis. The CIN phenotype may thus overcome at least the direct problems associated with aneuploidy.

CIN versus Aneuploidy on Genome Integrity

The consequence of aneuploidy for the genome is mostly limited to alterations in gene copy numbers. In stark contrast, mitotic errors can have extensive secondary effects on genome integrity (Figure 1B). Although the underlying causes are still unclear and probably not limited to one mechanism, laboratory CIN cancer cell lines show specific forms of mitotic errors known as lagging chromosomes and anaphase bridges [16–18]. These are chromosomes that are not migrating with either of the two packs of chromosomes during anaphase. As a result, they are trapped in the cleavage furrow or form a separate micronucleus in one of the daughter cells [46,47] (Figure 1B). Trapped chromosomes can acquire double-strand DNA breaks that are repaired by the error-prone nonhomologous end joining pathway in G_1, resulting in structural rearrangements such as translocations [46]. Such genomic rearrangements have also been observed in tumors of a CIN mouse model [48].

Micronuclei too can give rise to DNA damage and genomic rearrangements. The chromosomes in micronuclei suffer from replication stress and are additionally damaged when their fragile nuclear envelope collapses, exposing the DNA to the hostile cytoplasm [47,49]. The catastrophic chromosome fragmentation results in various kinds of genome rearrangements, including chromothripsis [50–52]. In the longer term, when cells harboring rearranged chromosomes are allowed to continue to proliferate, a subset of those cells may engage in a breakage–fusion–bridge cycle [53,54], thus creating a vicious cycle of chromosome missegregation and genome insults (Figure 1B, dashed lines).

While aneuploidy is a logical aberration in CIN cells, the reverse may also occur. Several studies have suggested that aneuploidy can predispose to additional aneuploidies, genome rearrangements, and increased mutational burden on the nucleotide level [37,55–59]. Aneuploidy may thus cause a CIN phenotype, adding another layer to the aforementioned vicious cycle. However, other studies have contradicted this [18,60] and the presence of stably aneuploid cancer cell lines (like HCT 116) implies CIN is not an obligatory outcome of aneuploidy.

CIN versus Aneuploidy on the Course of Tumor Evolution

Given that CIN cells frequently reshuffle their genomes and aneuploidy is a static state, the two are predicted to influence the evolutionary dynamics of a developing tumor in distinct ways. During tumorigenesis, CIN increases population plasticity by stochastically providing genomic variants that are subsequently enriched or purged via evolutionary dynamics (Figure 1A). Accordingly, CIN could be a promoter of tumorigenesis, and indeed, CIN can act to enhance or even initiate tumorigenesis in several mouse models [61] while stable aneuploidies impair embryonic development [36,62]. CIN mouse models usually have decreased mitotic fidelity due to germ-line mutations in genes that regulate mitosis. Therefore, elevated mitotic errors are present already from conception onward. Since CIN might have different influences on tumorigenesis in different tissues and at different points during tumor evolution, conditional CIN animal models will advance our understanding of CIN in tumor progression.

CIN levels in tumors are frequently inferred from karyotype analyses, which (as outlined earlier) cannot distinguish between ongoing mitotic errors and selection on karyotypes. Whether CIN is an early acquired phenotype of tumor cells and whether CIN is present throughout tumor evolution is therefore currently unknown. In fact, even the presence of aneuploidy in the tumor cannot speak to whether CIN is or has been present during the development of the tumor. The missegregation rate of healthy cells in 2D cell culture is estimated at approximately 0.025% per chromosome per division [18]. In certain instances, the aneuploid cells may remain in the population and can acquire more complex aneuploidies over time. *In silico* modeling has provided support for this, as aneuploidy tolerance in the absence of CIN was sufficient to explain the lower-grade aneuploidies observed in tumors, and CIN was only important to generate higher-grade aneuploidies [63]. Direct measurement of CIN will thus give answers regarding the presence of CIN in human tumors (Box 2).

Whereas a CIN phenotype provides the ability to continuously reshape genomic content, allowing adaptation to changing environmental conditions, aneuploidy enables at best only a temporary advantage. Predominant aneuploidies within a tumor reflect the favored oncogenic karyotypes resulting from mitotic errors. These aneuploid karyotypes are thus not only the result of mitotic errors, but also from selection. This is illustrated by a number of observations. First, tumors generally harbor dominant karyotypic traits shared by the majority of tumor cells, which were recently shown to be also true in a CIN mouse model [64]. Second, different tumor types are characterized by different karyotypic traits, implying that specific genes on these chromosomes exert tissue-specific oncogenic benefits. For example, intestinal tumors typically gain copies of chromosome 13q [65–67], and glioblastoma multiforme is generally characterized by gains of chromosome 7 and loss of 10 [68,69]. Such differences enable discrimination between tumors originating from different tissue lineages, as well as specific tumor types [70]. Third, predisposition to certain types of cancer in hereditary aneuploidy patients depends on the specific karyotype involved [71]. Finally, increasing aneuploidy levels are generally correlated with later tumor stages, for example, in breast [72] and colon cancer [73], suggesting that several rounds of selection accumulate different karyotypic changes during tumor evolution.

Box 2. Measuring Chromosomal Instability (CIN) and Aneuploidy

Assessment of aneuploidy and CIN status in tumor samples will be required to test feasibility of therapeutic options described here. Although aneuploidy status can relatively quickly be determined by various karyotyping or fluorescence *in situ* hybridization (FISH)-based methods, such techniques have several limitations. Karyotyping generally requires trapping cells in mitosis and is therefore biased to fast-cycling cells. FISH can be performed on interphase nuclei but can only analyze a limited number of loci. Recent advances in single-cell sequencing (SCSing) approaches will aid in overcoming such limitations. Practical and economic arguments currently preclude widespread use of SCSing, but this may be the approach of choice when such issues are overcome [97]. SCSing gives genome-wide information at single-cell resolution and thereby allows estimation of intratumor heterogeneity, and thus, possibly additional clues regarding therapy sensitivity.

Determining the level of CIN in clinical samples is more challenging, since the rate and level of errors during cell divisions need to be assessed. Although not ideal in terms of quantification of all parameters, visual inspection of anaphases in hematoxylin and eosin-stained tumor sections may be sufficient to determine the severity and rate of segregation errors. The generally low mitotic indices in tumors may, however, complicate such approaches. To truly determine CIN, visualization of chromosome segregation over subsequent generations is required. In fundamental research settings, this is most commonly achieved by fluorescent labeling of histones and subsequent live-cell imaging. Although doable with simple and affordable imaging systems, various practical reasons complicate its use for patient material. A potential solution is the use of nontoxic DNA dyes such as SiR-DNA that can be added directly to the sample prior to imaging [98]. Another challenge is the need for living tumor material. This may be facilitated by recent developments in culturing stem cell-based 'organoid' structures that develop *ex vivo* in membrane extract gels and in the presence of niche factors provided in the growth medium [99]. Organoids can be grown from tumors and retain the major characteristics of the original tumor, thus providing an excellent *ex vivo* intermediate model [100–104]. We have recently shown the onset of CIN in an artificial intestinal tumor progression organoid model [105], showing that imaging of mitotic errors in organoids is technically feasible. Improvements in low-cost high-resolution imaging and organoid cultures might make this type of approach a viable option for more widespread use in the future.

CIN versus Aneuploidy in Therapy Response and Relapse

Since therapeutic agents can be regarded as new selection pressures, CIN and aneuploidy might differentially influence therapy response and tumor relapse in ways similar to their impact on tumor evolution. The cellular plasticity provided by a CIN phenotype will therefore not only accelerate tumorigenesis, but also the outgrowth of resistant clones, and thus, tumor relapse. Aneuploidy, too, can have profound impact on therapy by similar mechanisms but with a different outlook after relapse and different options for combination therapies.

Let us consider two hypothetical tumors (Figure 2). Both are karyotypically heterogeneous and have moderate to high aneuploidy tolerance. One however contains predominantly CIN cells, while the other is mitotically stable. Note that both tumors show karyotype divergence, but that the divergence in the mitotically stable tumor is the result of evolutionary dynamics. When faced with a therapeutic agent (Figure 2, Drug A), most cells in both tumors will die but a small population will prove resistant as a result of certain karyotypes within that population. Heterogeneity in the surviving population will be greatly reduced. Interestingly, this population in the mitotically stable tumor is amenable to the 'evolution trap' approach, in which a second drug (Figure 2, Drug B) specifically targets the karyotype that survived the first agent [74]. All tumor

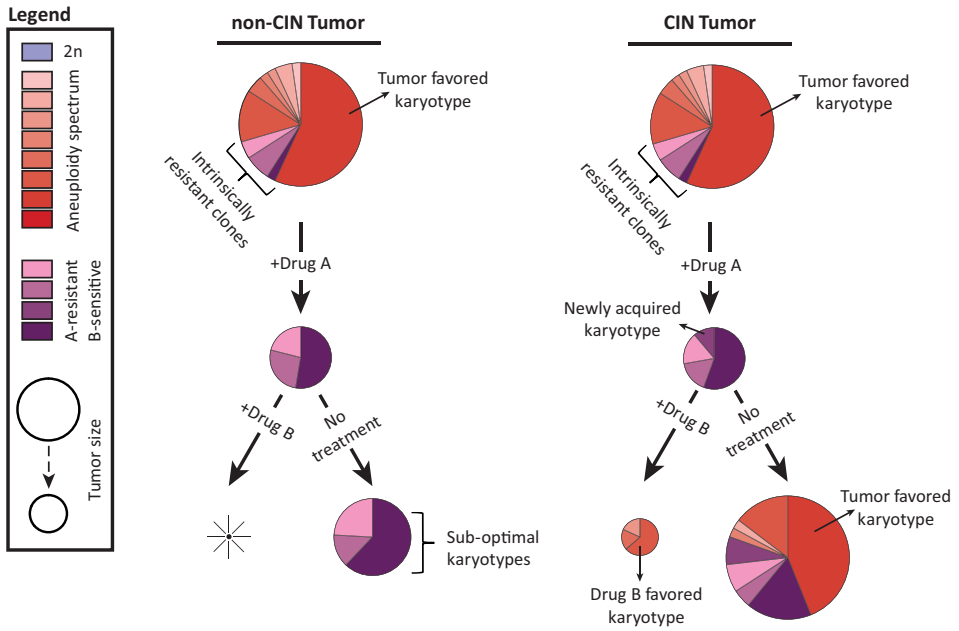

Figure 2. Influences of Chromosomal Instability (CIN) on Response to Therapy and Tumor Relapse. Two karyotypically identical tumors, one of which contains cells with the CIN phenotype (right side), are both treated with Drug A. Intrinsically resistant clones in each tumor will survive. In addition, CIN cells can generate new resistant karyotypes. The karyotypic characteristics that confer therapy resistance to Drug A simultaneously induce sensitivity toward Drug B. The tumor is now efficiently eradicated since only sensitive karyotypes populate the tumor, as these are selected for by Drug A. The CIN tumor cells, however, can generate new resistant karyotypes and so this tumor will again present resistant clones. If no second treatment is applied after Drug A, both tumors will relapse. In the non-CIN tumor, only the selected clones will regrow, whereas in the CIN tumor, new clones will be generated that can again gain the optimal karyotype for tumor growth. CIN can thus both enhance drug resistance and tumor relapse. Pie charts depicting cell populations; the colors reflect diploid (blue) or different aneuploid (shades of red) karyotypes; red karyotypes are sensitive to Drug A and resistant to Drug B; pink to purple colors depict cells with karyotypes that are resistant to Drug A and sensitive to Drug B.

cells are eventually eradicated by the second-line treatment. However, if the surviving cells are CIN, they are likely to generate novel resistant karyotypes (i.e., acquired resistance). The relapsed tumor, while potentially resensitized to the original drug, is not amenable to the evolution trap approach. Although the scenario delineated in the figure is at present purely theoretical, it illustrates the need for individual assessment of CIN, karyotype divergence, and aneuploidy when addressing such therapeutic approaches. In the given example, karyotype measurements will not suffice in selecting the right populations.

In addition to enhanced resistance, CIN potentially also accelerates tumor relapse. The non-CIN tumor will regrow with suboptimal karyotypes and is unable to quickly regain new karyotypes. The CIN cells in the other tumor can, however, accelerate the appearance of karyotypes optimal for tumor growth (Figure 2). This enhanced tumor relapse has been observed *in vivo*, where induction of CIN by overexpression of Mad2 accelerated tumor relapse in a Ras-withdrawal lung cancer model [75].

In summary, both CIN and aneuploidy can theoretically provide therapy resistance via evolutionary mechanisms. But whereas aneuploid populations may harbor intrinsic resistant clones, CIN additionally provides cellular plasticity and might thereby promote acquired resistance and tumor relapse.

CIN versus Aneuploidy in Choice of Therapy

Antimitotic agents such as vinca alkaloids and taxanes are important clinical cancer therapy drugs. Both types of drugs interfere with microtubule dynamics and are well-known inducers of mitotic delays. In 2D cultures, cells treated with taxol either undergo mitotic catastrophe or mitotic slippage [76]. A recent study examining the cytotoxic effects of paclitaxel in breast cancer reported that, at clinically relevant concentrations, the drug induces multipolar divisions [77]. Cells thus appeared to die as a result of too severe segregation errors (likely causing severe proteotoxicity and DNA damage, as outlined earlier). CIN cells may already have acquired substantial tolerance for segregation errors and as such may not be very sensitive to taxanes. In line with this, tumors deemed CIN by a CIN70 gene expression signature [78] were more resistant to taxanes [79], a correlation seen more widely [80]. It is unknown, however, if these tumors still made frequent mitotic errors, and it would be interesting to examine correlation between mitotic errors and taxane resistance.

Seemingly at odds with the observation that CIN tumors are more resistant to taxanes is the hypothesis, proposed by us and others, that CIN tumors might be sensitive to exacerbation of mitotic errors. The sensitivity may depend on the level of increase of CIN induced by the treatment and whether this exceeds an aneuploidy tolerance and/or DNA-damage-tolerance threshold. This concept is in concordance with hormesis: The situation when a low dose of an entity is beneficial for cellular fitness while a high dose is lethal (Figure 3). Indeed, CIN promotes tumor progression only at limited dose and is tumor suppressive at higher doses [81,82] and these findings are supported by stochastic modeling of karyotype evolution [83,84]. In addition, recent pan-cancer genome analysis suggested 'the existence of an optimal degree of genomic instability' [85]. Increasing CIN to high levels by combining an attenuated spindle checkpoint with low doses of taxanes or inhibition of the mitotic kinesin centromere protein E killed tumor cells of various kinds *in vitro* [86,87]. Inhibition of the spindle checkpoint was key in combination therapy studies, and drug discovery projects related to inhibition of TTK/Mps1, a crucial kinase in the spindle checkpoint, have been initiated by a number of companies. Various studies using xenograft models have verified that TTK/Mps1 inhibition can enhance the effect of taxol in treatment of, for example, triple-negative breast cancer [88], glioblastoma [89,90], and melanoma [91].

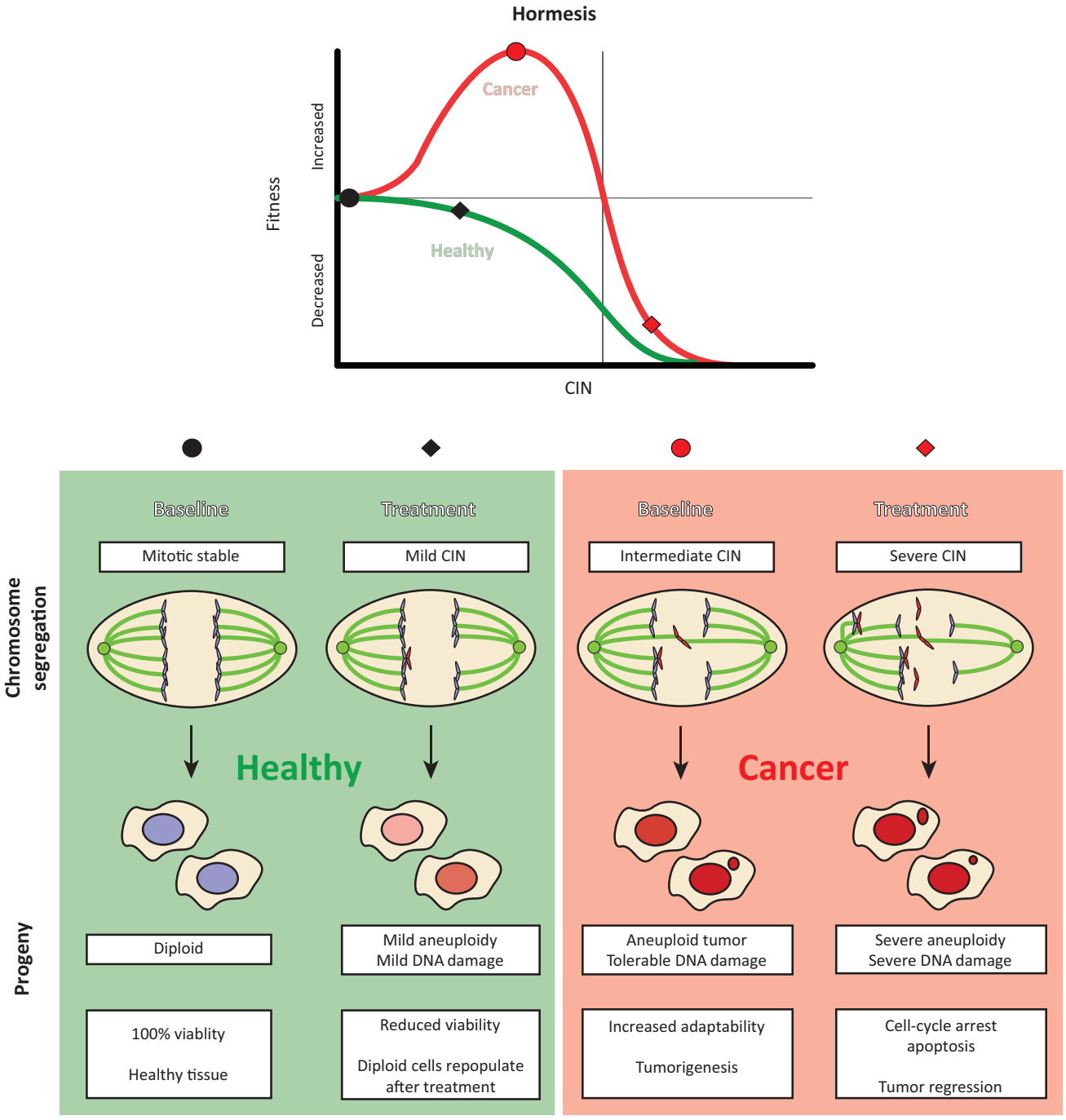

Figure 3. Enhancing Chromosomal Instability (CIN) as a Strategy to Kill CIN Cells. CIN probably acts according to hormesis in cancer cells, as illustrated by a hypothetical fitness curve (top). Whereas an intermediate level of CIN is beneficial for development of the tumor, higher levels will be detrimental for survival (red line). This benefit is likely absent in healthy cells (green line). Enhancing the baseline CIN level (filled circles) with a drug (filled diamonds) might be therapeutically beneficial. The increase of CIN in healthy cells will only mildly impair fitness of the progeny (green, left side), whereas increased mitotic errors in already CIN cells will generate severe aneuploidy and DNA damage and subsequent cell death (orange, right side). CIN levels are probably easily elevated in CIN cells, given they already contain intrinsic causes for missegregation, such as dicentric chromosomes as illustrated on the right. Correct segregating chromosomes and diploid cells are depicted in blue, missegregating chromosomes and aneuploid cells are in red.

Whether the CIN hormesis concept will hold in clinical settings remains to be seen. Importantly, the efficacy of this approach relies on the ability of a given treatment to enhance CIN. Although a wide variety of drugs are capable of this [92], efficacy will vary between patients. It will therefore be important to determine CIN status when evaluating the feasibility of this approach (Box 2). Enhancing CIN as a therapeutic strategy requires passing the 'hormesis threshold' and therefore CIN tolerance is an important factor. This tolerance is likely to consist of a combination of aneuploidy and DNA-damage tolerance. Interestingly, CIN tumors appear to be more sensitive to DNA-damaging agents [79] and radiation therapy [93], which might be explained by exceeding the DNA-damage component of CIN tolerance.

Aneuploidy may also be amenable to hormesis-based therapy, but by a different mechanism than CIN: By increasing the general stresses accompanying an unbalanced karyotype. Aneuploid cells tend to be more sensitive to inhibition of the pathways regulating proteostasis, like protein folding and autophagy, resulting in toxic overload of these pathways [94,95]. For a more elaborated view on aneuploidy as a therapeutic target, we refer interested readers to an excellent recent review [96].

Therapeutically exploiting aneuploidy will likely depend on the mechanism and level of aneuploidy tolerance in those cells. Since aneuploidy tolerance might be a bottleneck for increasing karyotypic divergence, low divergence might predict higher sensitivity toward such therapeutic strategies. They will obviously benefit from an absence of CIN (and thus, cellular plasticity) as elaborated on earlier. Aneuploidy targeting strategies are thus likely most successful in mitotically stable, aneuploid tumors that show little divergence, and thus, this hypothesis again highlights the importance of individual assessment of CIN, aneuploidy, and karyotype divergence.

Outstanding Questions

What are the main causes for chromosomal instability (CIN) in human tumors?

Do human tumors retain a CIN phenotype, and if so, when during oncogenic transformation does CIN occur and does severity of CIN change during tumor progression?

How do CIN and aneuploidy influence tumor aggressiveness and therapy response?

Can CIN and/or aneuploidy be exploited in cancer therapy?

How do cancer cells benefit from and circumvent problems associated with aneuploidy?

Concluding Remarks

Whole-genome sequencing efforts in the past years made clear that no tumor is the same. Major improvements in cancer therapy will thus come from carefully matching individual patients with specific drugs, an approach referred to as personalized medicine. In order to select the best therapy, there is a great need for biomarkers, that is, an identifiable, tumor-specific feature that can predict drug response.

Here, we underpin the notion that more systemic alterations, that is, CIN and aneuploidy, can impact on tumor evolution and therapy response. This in turn may provide guidance in therapy-of-choice selection, and prompt the development of therapies that exploit vulnerabilities presented by either one. A broad empirical evidence basis to translate these insights to the clinic is currently lacking, and will require more careful assessment of CIN and aneuploidy in various cancer samples (Box 2), and assessment of correlation between this and the differential responses to therapies. Finally, distinguishing CIN from aneuploidy in cancers may help to address the long-standing questions as depicted in the Outstanding Questions section.

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