Aneuploidy in health, disease, and aging

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Aneuploidy, an aberrant number of chromosomes, has been recognized as a feature of human malignancies for over a century, but compelling evidence for causality was largely lacking until mouse models for chromosome number instability were used. These in vivo studies have not only uncovered important new insights into the extremely complex aneuploidy–cancer relationship but also into the molecular mechanisms underlying proper and aberrant chromosome segregation. A series of diverse mouse models for the mitotic checkpoint protein BubR1 has provided evidence for a provocative novel link between aneuploidization and the development of age-related pathologies.

Introduction

Aneuploidy refers to a state in which the number of chromosomes in a cell is not an exact multiple of the haploid set. Chromosomal instability (CIN), on the other hand, defines a condition in which cells are unable to accurately segregate whole chromosomes (whole CIN [W-CIN]) or prone to structural chromosome rearrangements (structural CIN [S-CIN]), including translocations, deletions, and duplications of large parts of chromosomes (Ricke et al., 2008). CIN genes are commonly classified as genes that increase the rate of numerical and/or structural chromosome alterations when mutated (Michor et al., 2005). In the early 1900s, Theodor Boveri hypothesized that aneuploidy was a causal feature of human cancers. This long-standing hypothesis was difficult to test until the development of targeted approaches to genetically manipulate mice and the discovery of genes and mechanisms that act to prevent chromosome number instability. Although the relationship between aneuploidy and tumorigenesis is characterized by ever increasing complexity, aneuploidy-prone mouse models revealed that the effect of W-CIN on tumorigenesis is highly dependent on the gene that is defective, including its other cellular functions, the extent or nature of the gene defect, the affected tissue or cell type, and the context of other cancer gene mutations (Ricke et al., 2008). Studies designed to explore the role of BubR1 in cancer uncovered a surprising link between abundance of this mitotic regulator and the rate of aging (Baker et al., 2004, 2013). This provided a molecular entry point for studies on age-related aneuploidization and its potential role in tissue/organ degeneration. The impact of aneuploidization on physiological homeostasis seems negative, but accumulating evidence suggests that select tissues are subject to orchestrated aneuploidization as part of normal tissue development (Rehen et al., 2001; Duncan et al., 2012b). Here, we highlight the recent advances in understanding the physiological impact of aneuploidy and CIN using mouse models as well as the new mechanistic insights these studies provided into proper and aberrant chromosome segregation.

Mechanistic insights into CIN gene function and malfunction

Early attempts to understand the aneuploidy–cancer relationship were hampered by a lack of information about the molecular genetic basis of mitosis, which is believed to involve hundreds of genes (Stirling et al., 2011). Although much of what is currently known about the molecules and mechanisms that drive chromosome segregation originates from in vitro studies, mouse models have been invaluable tools for obtaining mechanistic information for various reasons. First, gene-targeted and transgenic mice offer a clean genetic system in which all cells are afflicted in the absence of confounding preexisting genetic aberrations. Second, gene expression can be up- or down-regulated in a graded fashion, which has helped uncover the multifaceted nature of several CIN genes. Third, knockin mutations targeting specific domains of certain mitotic regulators have been instrumental for delineating their modular functions. Fourth, CIN genes can be analyzed in a wide variety of cell types residing in their natural tissue context, allowing for the identification of any mechanistic diversity in the execution of mitosis between distinct cell types.

The novel mechanistic insights gained from mouse modeling are perhaps best exemplified by studies of the mitotic checkpoint gene Bub1 (Fig. 1), for which seven different targeted mutations (Jeganathan et al., 2007; Perera et al., 2007; Leland et al., 2009; Schliekelman et al., 2009; Ricke et al., 2012) and several transgenic strains have been created (Cowley et al., 2005; Ricke et al., 2011). For example, conditional knockout alleles for Bub1 uniquely demonstrated that premature centromeric separation is a consequence of mitotic checkpoint weakening.
Bidirectional deviations from normal Bub1 levels and inactivation of Bub1 enzymatic activity universally cause aneuploid cells to accumulate in mice. Analysis of the underlying mechanisms of chromosome missegregation in each mouse model has provided important new insights into the multifaceted nature of this mitotic regulator. For instance, in addition to confirming that Bub1 plays a critical role in kinetochore assembly and mitotic checkpoint activity, studies of Bub1 hypomorphic mice (top) revealed that Bub1 acts as a crucial trigger to induce cell death after chromosome missegregation (Jeganathan et al., 2007). On the other hand, transgenic mice that overexpress Bub1 (middle) revealed the novel concept of Aurora B hyperactivity and linked it to chromosome missegregation (Ricke et al., 2011). Unlike Bub1 hypomorphic or transgenic mice, mice lacking Bub1 kinase activity (bottom) harbor significant aneuploidy without a predisposition to cancer. In this model, it was revealed that accumulation of Aurora B at inner centromeric regions is mediated by Bub1-mediated histone H2A phosphorylation at T121 in a shugoshin-independent manner (Ricke et al., 2012). P, phosphorylation.

Studies of various Mad2 mutant mice indicate that differential effects imparted by bidirectional deviation of protein expression may be a more common feature of mitotic regulators. Both up- and down-regulation of Mad2 predispose to aneuploidy, with Mad2 haploinsufficiency weakening mitotic checkpoint signaling and Mad2 overexpression hyperstabilizing microtubule–kinetochore attachments (Michel et al., 2001; Kabeche and Compton, 2012). On the other hand, bidirectional deviation of BubR1 results in divergent effects on aneuploidy, with BubR1 overexpression providing protection against aneuploidy and BubR1 insufficiency perturbing accurate chromosome segregation (Baker et al., 2004, 2013). That Mad2 and BubR1 overexpression have opposite effects on chromosome segregation is intriguing given that both function in a complex to inhibit anaphase-promoting complex (APC)/cyclosomeCdc20 (Kulukian et al., 2009). The divergence may simply reflect potential differences in level of overexpression or fundamental differences in protein function.

A key advantage of using mouse models to decipher the mechanisms by which CIN genes operate is that gene malfunction can be directly correlated to effects on health and disease. This has been particularly important to advance our insight into the intricate aneuploidy–cancer relationship.
Aneuploidy and cancer

Although aneuploidy has been long recognized to be a defining feature of cancer cell genomes, inferring the significance of chromosomal aberrancies in tumorigenesis has remained a challenge. Whereas some researchers argue that aneuploidy is a primary force driving tumorigenesis (Duesberg et al., 1998), others contend that aneuploidy is simply a side effect of malignant transformation (Zimonić et al., 2001). The confusion is in part caused by the heterogeneous nature of tumors, the broad landscape of genetic mutations a cancer cell harbors to thwart protective pathways (Wood et al., 2007), and the observation that few solid tumors undergo identical CIN events (Mitelman, 2000). Here, we first recognize the tremendous complexity of the cancer–aneuploidy issue, then discuss the various lines of evidence from mouse models that aneuploidy drives cancer, and finally provide an alternative look at the aneuploidy–cancer connection.

Multilevel complexity of the aneuploidy-cancer relationship. In addition to an incomplete understanding about the molecular genetic basis of mitosis, there are at least seven more layers of complexity regarding the actions of aneuploidy and CIN in human cancer.

1. Recurrent chromosome gains/losses are rare in human cancers. Although specific chromosome translocations often classify hematologic malignancies (Mitelman, 2000), recurrent gains/losses of specific chromosomes are extremely rare in any human cancer type, complicating the interpretation of whether whole chromosome reshuffling is crucial or irrelevant. Recent studies suggest that chromosome reshuffling in human tumors is not entirely arbitrary (Ozery-Flato et al., 2011; Duijff et al., 2013), but the role of co-occurrence of losses or gains of specific chromosomes in tumor evolution remains entirely unclear.

2. Inconsistency in aneuploidy measurement and interpretation. A database of published karyotypic abnormalities found in neoplastic diseases, now containing ~60,000 cases (Högland et al., 2002), is available to study the prevalence and frequency of karyotypes among tumor types. However, key challenges remain in analyzing the available information, including the lack of uniformity in data collection, the overreliance on metaphase spread karyotyping (which biases toward proliferating cells), and the limited knowledge about the degree of intratumor karyotypic heterogeneity (McGranahan et al., 2012).

3. Challenges in measuring CIN. CIN has been proposed to facilitate tumor adaptation (Gutenberg et al., 2010; Lee et al., 2011) and is a predictor for poor prognosis and treatment refractory tumors (Carter et al., 2006; Bakhoun et al., 2011; Birkbak et al., 2011). Despite these clinical implications, few methods measure the dynamic nature of CIN in tumors. One exception is FISH, which infers CIN from intratumor variation of chromosome copy number. A surrogate assessment for CIN is its molecular gene signature, as the total transcripational activity of a tumor can be reflective of unbalanced chromosome load, and chromosomally unstable tumors often aberrantly express chromosome integrity regulators (Upender et al., 2004; Carter et al., 2006; Gao et al., 2007; Pavelka et al., 2010).

4. The integral link between aneuploidy and W-CIN. Several studies provide evidence for a vicious cycle in which chromosome number imbalances undermine faithful chromosome segregation, causing further aneuploidization. The most compelling evidence is that certain aneuploid yeast strains are prone to additional karyotypic changes (St Charles et al., 2010; Sheltzer et al., 2011; Zhu et al., 2012). Consistent with this notion, some cells from humans with autosomal trisomies gain or lose other chromosomes at elevated rates compared with cells from diploid individuals (Amiel et al., 2006; Reish et al., 2006, 2011).

5. Temporal importance of CIN in tumors. Theoretically, CIN can emerge and act throughout the entire tumor process. However, the aneuploidy status of mature tumors provides little information about the timing and impact of numerical chromosome changes during tumor evolution. For instance, CIN occurring early during tumorigenesis may be masked by late-stage genetic alterations promoting karyotypic stability.

6. An apparent inseparable nature of W-CIN and S-CIN. Several lines of evidence suggest that impaired mitotic fidelity creates DNA damage that adversely impacts genome integrity. Structural chromosomal damage may occur when lagging chromosomes are trapped in the cytokinesis furrow (Janssen et al., 2011). Alternatively, micronuclei formation caused by lagging chromosomes may drive loss of structural integrity through breakage–fusion–bridge cycles or the more extreme process of chromosome pulverization (Guerrero et al., 2010; Crasta et al., 2012). The latter process may explain the phenomenon of “chromothripsis,” during which chromosomes undergo extensive rearrangements (Hastings et al., 2009; Liu et al., 2011; Stephens et al., 2011).

Evidence for causality. Three independent lines of evidence from mouse models support the hypothesis that there is a causal relationship between aneuploidy and tumorigenesis. First, if aneuploidy were a causal feature of tumorigenesis, one would expect that increasing aneuploidization in mice would increase tumor predisposition. Indeed, most of the several dozen chromosomally unstable mouse models are tumor prone (Pfau and Amon, 2012). This includes mice with aneuploidies in mitotic checkpoint signaling (Michel et al., 2001; Iwanaga et al., 2007; Jeganathan et al., 2007; Weaver et al., 2007; Li et al., 2009; Schliekelman et al., 2009), centrosome duplication (van Ree et al., 2010), spindle assembly (AguiRrue-Portolés et al., 2012; Zhang et al., 2012), microtubule–kinetochore attachment (Solitio et al., 2007; Weaver et al., 2007; Diaz-Rodríguez et al., 2008), or attachment error correction (Fernández-Miranda et al., 2011; Ricke et al., 2011), suggesting that tumor propensity is independent of the mechanism driving the aneuploidy. As in vitro studies have linked aberrant chromosome segregation to structural chromosomal abnormalities (Guerrero et al., 2010; Janssen et al., 2011; Crasta et al., 2012), it will now be important to carefully analyze the available W-CIN models for evidence of S-CIN predisposition.
Second, if aneuploidy was a driving force in tumorigene-
sis, one might predict that protection against aneuploidi-
zation would attenuate tumor formation. One mouse model that
suppresses chromosome missegregation is a transgenic mouse strain
that overexpresses the mitotic checkpoint protein BubR1. Indeed,
spontaneous, carcinogenic, and genetically induced tumorigene-
sis are all reduced in these mice (Baker et al., 2013). BubR1 is
unique in that its overexpression protects against aneuploidy, as
overexpression of other mitotic regulators, such as Bub1, Mad2,
UbcH10, and Hec1, increases aneuploidyization (Sotillo et al.,
2007; Diaz-Rodriguez et al., 2008; van Ree et al., 2010; Ricke
et al., 2011). That BubR1 overabundance protects against aneu-
plodiﬁcation is remarkable considering that overexpression of
Mad2, a mitotic regulator that similarly acts to prevent precoc-
ious APC/cyclosome activation, induces aneuploidy through hy-
perstabilization of microtubules to kinetochores (Sotillo et al.,

Third, genetic alterations that promote chromosome misseg-
regation have long been proposed to drive tumorigenesis through
loss of whole chromosomes containing key tumor suppressor
genes. Speciﬁcally, it has been shown that whole chromosome
misregulation, caused by Bub1 hypomorphism, promotes loss of
heterozygosity to potentiate tumorigenesis in two different
mice with tumor suppressor backgrounds, p53 and APC (Baker et al.,
2009; Baker and van Deursen, 2010). Whether this is a universal
feature of CIN and what contexts drive these key events remain unclear,
as Bub1 hypomorphism initiates the loss of key tumor suppressors in restricted genetic contexts. Moreover, aneuploidy driven
by haplinsufﬁciency of either Bub1 or Bub3 was unable to pro-

come p53 loss of heterozygosity (Kalitsis et al., 2005; Baker et al.,
2009). Therefore, understanding which CIN genes cooperate to
promote this type of event will require further clarity.

Although many W-CIN mouse models are tumor prone, why
some mouse strains with CIN are susceptible to tumorigen-
esis and others are not remains a key unanswered question. Cu-

ciously, the incidence of tumorigenesis does not correlate with
aneuploidy levels, although technical limitations preclude a sys-

tematic, animal-wide analysis of aneuploidy. Additionally, aneu-
plodiﬁcation in proliferating cells, measured using karyotyping,
may not be representative of nondividing cells, measured using FISH,
particularly if aneuploidy prevents proliferation in those tissues
(Torres et al., 2007; Williams et al., 2008). Tumor spectrum is
also independent of the error driving aneuploidy. For example,

mice with chromosome instability caused by DNA replica-
tion defects develop tumors with a similar spectrum as canonical
W-CIN mice (Chuang et al., 2010; Kawabata et al., 2011). Simi-
larly, mice with aneuploidy as a result of a variety of mitotic de-
fects develop similar tumor types (Jeganathan et al., 2007;
Schliekelman et al., 2009; van Ree et al., 2010; Fernández-Miranda
et al., 2011; Ricke et al., 2011; Zhang et al., 2012). This implies
that the stochastic nature of aneuploidiﬁcation is sufﬁcient to drive
the tumor process rather than any speciﬁc activities.

A hierarchical view of the aneuploidy-cancer
connection. Studies on the molecular genetic basis of chro-

some segregation have largely centered on understanding the
workings of various mitotic processes, including chromosome
condensation, kinetochore assembly, spindle formation, spindle
pole migration, microtubule–kinetochore attachment, nuclear
envelope breakdown, mitotic checkpoint activation, and attach-
ment error correction (Fig. 2). Here, we classify components
acting in these processes as direct mitotic regulators. However,
increasing evidence suggests that various cellular processes oc-
curring outside of mitosis can be key determinants of segregation
accuracy. We deﬁne components implicated in these nonmitotic
processes as indirect mitotic regulators (Fig. 2). For example, cells
with supernumerary centrosomes demonstrate an increased fre-
quency of lagging chromosomes during anaphase, resulting from
aberrant microtubule–kinetochore attachment before centrosome
clustering and anaphase onset (Ganem et al., 2009; Silkworth
et al., 2009). Moreover, incomplete DNA duplication combined
with precocious mitotic entry has been proposed to drive anaphase
bridges or lagging chromosomes (Chan et al., 2009; Kawabata
et al., 2011; Remeseiro et al., 2012). Although incomplete DNA
replication results in the linkage of sister chromatids, improper
resolution of other forms of topological linkages, such as DNA
catenation and cohesin ring assembly, may impair chromosome
segregation fidelity (Diaz-Rodriguez et al., 2008; Xu et al.,
2010; Solomon et al., 2011; Remeseiro et al., 2012). Finally, proteins that regulate transcriptional or posttranslational expression of mitotic regula-
tors may indirectly impact mitotic fidelity. One example is Mad2,
whose expression is controlled by retinoblastoma (Rb) and E2F,
the p53–p21 pathway, and the E3 ubiquitin ligase SCF
(Varshavsky et al., 2002; Hernando et al., 2004; Guardavaccaro
et al., 2008; Manning and Dyson, 2011; Schwartzman et al.,
2011).

From a basic science perspective, it is important to under-
stand how each of the several hundred direct/indirect mitotic
regulators contribute to the accuracy of chromosome segregation.
From a cancer biology perspective, it is imperative to identify the
CIN genes that are altered in human tumors and to deter-
mine which of these gene alterations drive neoplastic transfor-
mation (Fig. 2). Unfortunately, our knowledge about both the
identity and the effects of CIN genes altered in human malign-
ancies is very limited. Gene expression proﬁles that predict CIN
status and treatment outcome could assist these eﬀorts. One such
proﬁle consists of 70 aberrantly expressed genes, referred to as
CIN70, many of which are implicated in DNA replication or
chromosome segregation (Carter et al., 2006). An alternative
signature of 11 overexpressed genes associated with tumor ag-
gressiveness and poor prognosis is enriched for mitotic factors,
including CcnB1, Bub1, and Hecl/Ndc80 (Glinsky et al., 2005).
Animal modeling will be instrumental in discriminating between
alterations in CIN gene expression that represent true oncogenic
events compared with alterations simply caused by the increased
proliferative index that tumors have.

Based on currently available data from mouse modeling, we
envision that CIN gene alterations that are found in human
cancers and induce aneuploidiﬁcation will fall into one of three
classes (Fig. 2). First, the particular CIN gene defect found in
human cancers counteracts tumorigenesis, such as observed for
Bub1 overexpression (Baker et al., 2013). Another example is
Bub1 hypomorphism, which besides aneuploidy promotes se-
nescence, a widely recognized antitumor mechanism (Baker
et al., 2008; Rodier and Campisi, 2011). Second, the CIN gene
aberrancy has little or no impact on tumorigenesis, as is the case
potentiate aneuploidy, perhaps through deregulated E2F activities, which target S- and M-phase genes, including Mad2 (Zheng et al., 2002; Hernando et al., 2004; Baker et al., 2009; Conklin et al., 2012). In vitro oncogenic Ras directly perturbs the accuracy of mitosis before transformation (Denko et al., 1994; Woo and Poon, 2004; Baker et al., 2013), potentially by deregulating prometaphase (Sarthy et al., 2007; Luo et al., 2009). Thus, these CIN genes could represent the top of the aneuploidy hierarchy by driving tumorigenesis through multiple mechanisms.

Aneuploidy during development and aging

Besides cancer, aneuploidization has recently been linked to two other physiological processes, development and aging. Indeed, in select tissues, such as brain and liver, aneuploidization seems to be an integral part of normal organ development (Rehen et al., 2001; Kingsbury et al., 2005; Duncan et al., 2012b), raising the intriguing concept that aneuploidy in some settings may not be detrimental and perhaps even be beneficial. The emerging connection between aneuploidy and aging is particularly fascinating,
as aging is known to be the main risk factor for chronic diseases and declining health. In this section, we first review the novel concept of orchestrated aneuploidization during development and then the provocative link between aneuploidization and the development of age-related pathologies.

**Orchestrated aneuploidization.** Studies designed to understand the development and function of the mammalian central nervous system revealed aneuploidization in a significant proportion of normal human and mouse brain cells, including mitotic cells and postmitotic neurons (Rehen et al., 2001, 2005; Yang et al., 2003; Kingsbury et al., 2005). The biological relevance of aneuploidization in the developing and mature brain remains speculative. One theory is that aneuploidies promote cellular diversity in the brain, thus perhaps contributing to the plasticity necessary for complex functions such as learning and memory (Kingsbury et al., 2006; Faggioli et al., 2012). Hepatocytes are also subject to orchestrated aneuploidization. They first become polyploid and then undergo reductive division, a process characterized by massive chromosome loss and the creation of near-diploid aneuploid cells (Duncan et al., 2009, 2010, 2012b; Faggioli et al., 2011; Gentric et al., 2012). It has been suggested that this process may grant the tissue a selective advantage to guard against varied and unknown assaults (Duncan et al., 2012a).

A key open question is whether orchestrated aneuploidy as part of a developmental process applies to tissues other than liver and brain. It will also be important to further explore the molecular mechanisms and functional implications of orchestrated aneuploidy, as studies into the adjustment of neurons and hepatocytes to chromosome imbalances may provide novel insights into the cellular responses to aneuploidy. One possibility is that each specific cell type buffers against the adverse effects of aneuploidy by regulating the expression of detrimental aneuploidy-induced targets. Alternatively, chromosome-specific events may allow the accumulation of certain gene products that provide cells with an advantage for a particular phenotype.

**Aneuploidy and accelerated aging.** Age-related aneuploidization has been well documented for oocytes and is considered to be the main cause of female reproductive infertility (Nagaoka et al., 2012). Men are known to be subject to age-related loss of the Y chromosome in several tissues, but the physiological impact of this phenomenon has remained unclear (Jacobs et al., 1963; Pierre and Hoagland, 1972). Initial evidence for a connection between regulators of chromosome segregation and somatic aging was provided by a study designed to investigate the aneuploidy–cancer relationship through a series of mice with graded reduction in BubR1 (Baker et al., 2004). Mutant mice carrying two hypomorphic BubR1 alleles and expressing ~10% of normal BubR1 levels were prone to aneuploidy as anticipated but, surprisingly, instead of tumors, developed a series of progeroid and age-related pathologies including short lifespan, sarcopenia, growth retardation, cataracts, fat loss, impaired wound healing, and reduced dermal thickness (Fig. 3; Baker et al., 2004).

Studies on individuals with a rare human recessive autosomal disorder called mosaic variegated aneuploidy (MVA) syndrome have subsequently reinforced the link between BubR1 insufficiency and progeroid disease (Hanks et al., 2004; Matsuura et al., 2006). MVA is a pediatric syndrome implicated in the literature as a hereditary cancer syndrome based on increased risk for childhood cancers such as rhabdomyosarcoma, Wilms’ tumor, and leukemia (Limwongse et al., 1999; Hanks et al., 2004, 2006; Matsuura et al., 2006; García-Castillo et al., 2008). However, MVA is a poorly characterized heterogeneous disease that can also be classified as a progeroid syndrome based on features such as short lifespan, growth retardation, facial dysmorphism, and cataract formation. The majority of MVA patients have mutations in *BUBR1*, either biallelic mutations with one allele harboring a missense mutation and the other a nonsense mutation or monoaletic mutations combined with allelic variants producing low amounts of wild-type BubR1 (Hanks et al., 2004; Matsuura et al., 2006).
Overall, BubR1 protein levels are typically very low in patients with \textit{BUBR1} mutations, largely because mutant BubR1 proteins produced by these alleles tend to be unstable (Suijkerbuijk et al., 2010).

Mice that are doubly haploinsufficient for the mitotic checkpoint genes \textit{Bub3} and \textit{Rae1} (Babu et al., 2003) represent a second aneuploidy-prone mouse strain with an accelerated aging phenotype, although the rate of premature aging is less profound than in \textit{BubR1} hypomorphic mice (Baker et al., 2006). However, the myriad of other aneuploidy mouse models have not been reported to exhibit early traits of early aging. At the surface, this argues against the idea that aneuploidy is sufficient to accelerate aspects of the aging process, but this may be premature for several reasons. First, most aneuploidy models were generated for the purpose of studying cancer predisposition, with mice typically being sacrificed between 14 and 18 mo to thoroughly screen for tumors. Thus, most of these studies would have missed accelerated aging phenotypes that develop later in life but nonetheless prematurely. Second, age-related deterioration may not be overt in most aneuploid models or may be restricted to select tissues. Such was the case for mice harboring one engineered allele that mimics a \textit{BubR1} nonsense mutation found in MVA patients with biallelic \textit{BubR1} mutations. In depth analyses of this model revealed shortened lifespan and accelerated onset of sarcopenia, cataracts, and fat loss (Wijsshake et al., 2012). Third, not all MVA patients have mutations in \textit{BubR1}, implying that other genes are linked to this progeroid syndrome. One such candidate is \textit{Cep57}, a gene encoding a centrosomal protein, which is mutated in a subset of MVA patients (Snape et al., 2011). Therefore, a thorough exploration of other aneuploidy-prone models is needed to determine the impact on aneuploidy on a broad range of tissues.

A likely possibility is that the aneuploidy and aging relationship is as complex as aneuploidy and cancer, such that there exists a hierarchy of CIN genes that also contribute to aging. Perhaps, aneuploidy-associated genes that are strongly linked with early aging, such as \textit{BubR1}, have multiple functions in preventing tissue deterioration. For example, BubR1 could counteract both aneuploidization and cellular stresses that engage senescence response pathways (Naylor et al., 2013). Consistent with this idea, the principal biomarker for senescent cells, p16\textsuperscript{ink4a}, is expressed at elevated levels in \textit{BubR1} progeroid mice (Baker et al., 2008). Clearing of these p16\textsuperscript{positive} cells, genetically or pharmacologically, delays progeroid features (Baker et al., 2008, 2011), providing a crucial link between senescence and aging. Clearly, a thorough evaluation of other aneuploidy-prone models is needed to determine the impact of aneuploidy on a broad range of tissues. For this, a system level approach may be useful to screen for phenotypic alterations in a variety of tissues (Guan et al., 2012).

\textbf{Aneuploidy and natural aging.} The link between BubR1 and early aging raises the question as to whether BubR1 is implicated in natural aging. One observation consistent with such a role is that BubR1 levels decline in various tissues with chronological aging, at least in mice (Baker et al., 2004, 2008). The underlying mechanisms are poorly understood and may occur at both transcriptional and posttranslational levels. BubR1 expression could simply decline as a result of reduced cell proliferation with aging, but a study on transgenic mice that constitutively overexpress BubR1 and are not subject to an age-related drop in BubR1 seem to argue against this (Baker et al., 2013). BubR1 transgenic mice live longer than normal mice and have an increased healthspan (the period during which an organism is free from serious or chronic disease, including cancer) characterized by attenuated muscle and renal atrophy, glomerulosclerosis, and increased cardiac function.

These studies further uncovered that aneuploidization is a hallmark of aging (Baker et al., 2013), raising the possibility that age-related aneuploidy contributes to tissue dysfunction. Consistent with this idea, reduced senescence and tissue deterioration in \textit{BubR1} transgenic mice tightly correlated with attenuated age-related aneuploidy (Baker et al., 2013). How BubR1 overexpression counteracts chromosome missegregation remains under investigation, with early evidence suggesting that defects in mitotic checkpoint control and microtubule–kinetochore attachment are ameliorated (Baker et al., 2013). This would imply that both these mitotic processes are subject to age-related decline and at least partially responsible for age-related aneuploidy. Interestingly, the degree of aneuploidization with aging tissue is dependent on proliferative index, as highly proliferative tissues and stem cells show relatively low rates, and largely postmitotic tissues demonstrate higher rates (Baker et al., 2013). One potential explanation is that tissues and cell types with an increased proliferation index are inherently more protected against chromosome segregation than cells that occasionally proliferate. Alternatively, euploid cells may outcompete aneuploid cells in highly proliferating tissues because of the antiproliferative influence of aneuploidization (Williams et al., 2008).

In Fig. 4, we have presented a hypothetical model for how aneuploidization might modulate health- and lifespan based on the available data from wild-type mice and the various models of accelerated and attenuated aneuploidy. It is important to note that aneuploidy is not the only age-related stress and that the effects of varying aneuploidy rates on tissue and organ deterioration have to be considered in the context of a variety of other aging-related stresses. It will be important to further test this provocative model in future experiments.

\textbf{Conclusions and future studies} The burst of animal modeling that started over a decade ago to critically test Boveri’s theory has provided compelling evidence that CIN provides selective pressure to initiate and propagate malignant transformation. However, the biological consequences of aneuploidy are clearly not limited to tumorigenesis, as aneuploidy correlates also with age-related tissue degeneration and rather paradoxically with benign gain-of-function processes such as in neural and liver cells. Thus, one important unifying theme emerging from the animal studies is the heterogeneity of phenotypes for both cancer and aging among the animals with different CIN gene defects. Perhaps the proposed hierarchical view of CIN genes that takes into consideration functions of these genes outside of mitosis may facilitate future studies aimed at deciphering the basis for this heterogeneity.
complexities in the biological outcomes induced by aneuploidy, these studies also signal that innovative and fresh perspectives are required to shed new light on the potential physiological role of aneuploidy. Finally, the only known gene alteration that counteracts aneuploidization and tumorigenesis in the absence of any overt adverse effects is BubR1 overexpression. Understanding how BubR1 exerts its beneficial effects at a modular level may provide important entry points for the design of small molecule-based therapies mimicking the effects of high BubR1.

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References


Babu, J.R., K.B. Jeganathan, D.J. Baker, X. Wu, N. Kang-Decker, and J.M. van Deursen. 2003. Ral1 is an essential mitotic checkpoint regulator that...


