Aneuploidy: Instigator and Inhibitor of Tumorigenesis

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Abstract

Aneuploidy, an aberrant chromosome number, has been recognized as a common characteristic of cancer cells for more than 100 years and has been suggested as a cause of tumorigenesis for nearly as long. However, this proposal had remained untested due to the difficulty of selectively generating aneuploidy without causing other damage. Using *Cenp-E* heterozygous animals, which develop whole chromosome aneuploidy in the absence of other defects, we have found that aneuploidy promotes tumorigenesis in some contexts and inhibits it in others. These findings confirm that aneuploidy can act oncogenically and reveal a previously unsuspected role for aneuploidy as a tumor suppressor. [Cancer Res 2007;67(21):10103–5]

Background: The Aneuploidy Controversy

Chromosome missegregation leading to aneuploidy was identified as a recurrent defect in many types of cancer cells in the late 1800s (1). Because of these findings, as well as his own observations of the pathologic consequences of chromosome missegregation, Theodor Boveri proposed aneuploidy as a cause of cancerous transformation in 1902 (2) and again in 1914 (3). This proposal, known as the aneuploidy hypothesis, has been staunchly supported by some (4, 5). However, the discovery of oncogenes and tumor suppressors in the late 1970s and 1980s introduced alternative potential initiators of transformation and resulted in reduced interest in the aneuploidy hypothesis. Some, favoring the importance of oncogenes and tumor suppressors, have argued against a role for chromosomal instability as a driving force in tumorigenesis (6). Others have argued that aneuploidy is only a benign side effect of transformation (7). Still others have suggested that aneuploidy promotes tumor progression but not initiation (8).

The controversy about the role of aneuploidy in tumorigenesis has stemmed from the inability to test the effects of aneuploidy in the absence of other defects. Most aneuploidy-inducing drugs have also been shown to cause additional affects, most notably DNA damage (9), which itself has been causally linked to tumor initiation (10). In the absence of a definitive test of the effects of aneuploidy, research has focused on the numerous associations between aneuploidy and precancerous lesions, including those of the cervix, head and neck, colon, esophagus, and bone marrow (11). Additionally, aneuploidy has been characterized as an indicator of poor prognosis (12). However, no causal link between aneuploidy and tumorigenesis can be made based on these observations.

Some attempts to address the role of aneuploidy in tumorigenesis have come from experiments using animals with reduced expression of mitotic checkpoint genes, including *Mad1, Mad2, BubR1*, and *Bub3*. The mitotic checkpoint (also known as the spindle assembly checkpoint) is the major cell cycle control mechanism that acts during mitosis to prevent chromosome missegregation and aneuploidy. Complete deletions of mitotic checkpoint genes are uniformly lethal in mammals, but animals with reduced expression of these proteins survive and develop aneuploidy at elevated rates (13–15). In some, but not all cases, these animals are more susceptible to spontaneous tumors. For instance, aged (≥18 month olds) mice heterozygous for *Mad1* develop a variety of benign and malignant tumors, whereas aged mice heterozygous for *Mad2* develop benign lung adenomas (15). However, aneuploidy due to reduction in BubR1 or Bub3 does not lead to an increase in spontaneous tumorigenesis (14, 16, 17). These experiments are complicated by the fact that all of these genes are expressed throughout the cell cycle and participate in multiple cellular functions. Mad1 and Mad2 bind to nuclear pores, where Mad1 functions in nuclear transport (18, 19). Mad2 participates in the DNA replication checkpoint (20) and Bub3 is a transcriptional repressor (21). BubR1 is involved in several cellular processes, including aging (14), apoptosis (22), megakaryopoiesis (23), and the response to DNA damage (24). Mad2, BubR1, and Bub3 have all been implicated in gross chromosomal rearrangements in yeast (25). Therefore, these genetically sophisticated attempts at dissecting the role of aneuploidy in tumorigenesis suffer from the same deficiencies as earlier experiments in that they examine the effects of aneuploidy only in the context of additional, often incompletely characterized, defects.

More recently, the mitotic checkpoint gene *Mad2* has been overexpressed in mice using a tetracycline-inducible approach. As suggested from the yeast data, cells overexpressing Mad2 develop a large number of chromosome breaks, fragments, and fusions in addition to whole chromosomal aneuploidy. This combination of DNA damage and aneuploidy, along with the other potential effects of Mad2 overexpression, leads to a large increase in spontaneous tumors, including adenomas of the lung, hepatomas, and intestinal tumors (26). Because reduction in the retinoblastoma tumor suppressor has been shown to lead to overexpression of Mad2 (27), this experiment has significant clinical relevance. However, because aneuploidy caused by Mad2 overexpression occurs in the context of additional defects, it does not offer a direct test of the effects of whole chromosome aneuploidy on tumor initiation or progression.

Resolution of the Aneuploidy Controversy: Aneuploidy Acts Both Oncogenically and as a Tumor Suppressor

We recently identified a method to generate aneuploidy without producing additional defects. Cells and animals heterozygous for the centromere-linked, kinesin-like motor protein CENP-E missegregate one or a few whole chromosomes at elevated rates during mitosis. Chromosome segregation errors in cells with reduced CENP-E are due to a weakened mitotic checkpoint (28) and impaired interactions between the chromosomes and the microtubules

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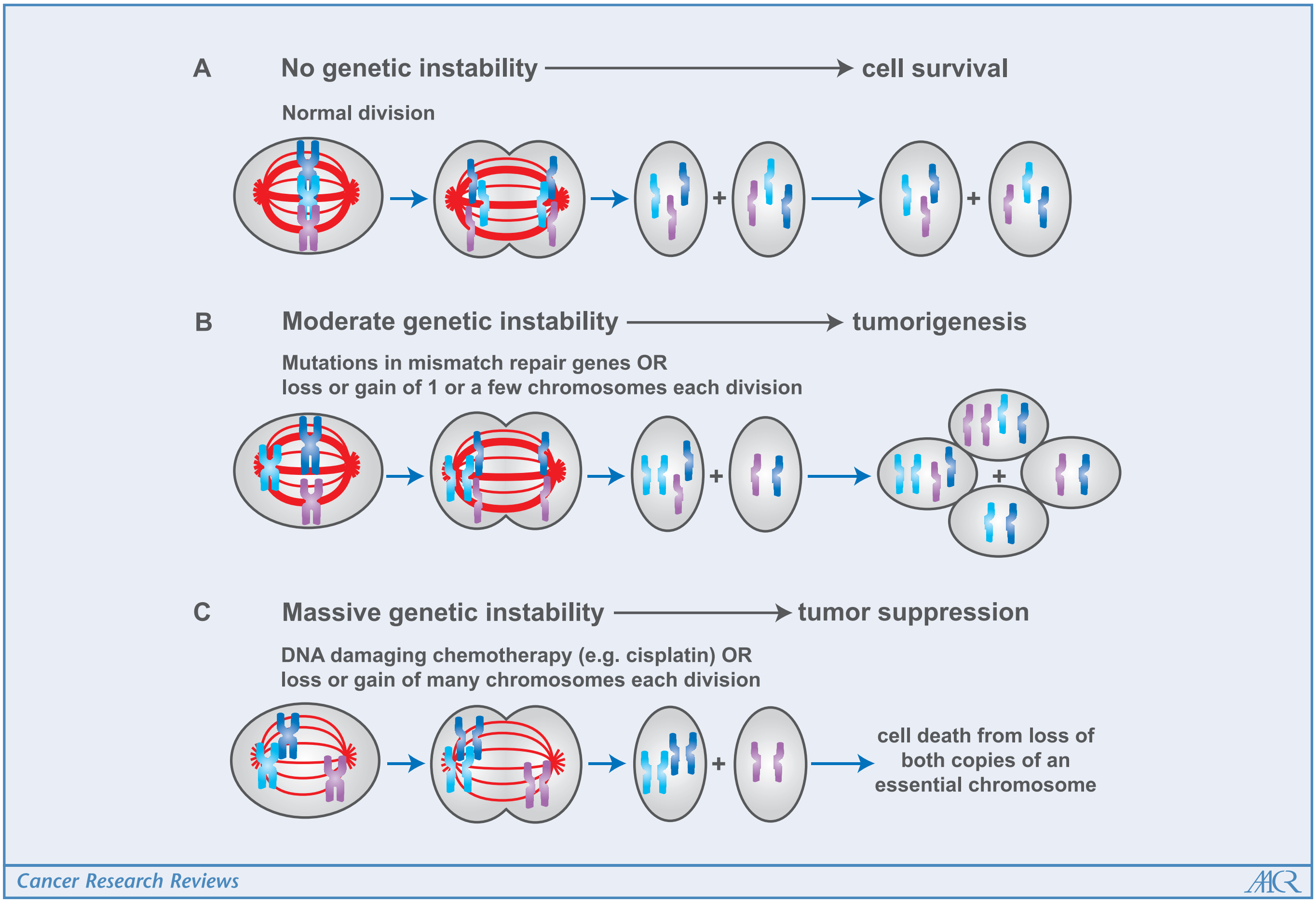

Figure 1. Aneuploidy can drive or inhibit tumors, similar to DNA damage. Wild-type cells do not exhibit genetic instability and maintain a diploid genome with intact growth-regulatory pathways, consistent with continued cell survival (*A*). Moderate levels of genetic instability, caused by mutations in mismatch repair genes or by missegregation of one to three chromosomes per division (due, for instance, to *Cenp-E* heterozygosity in the absence of other defects), promote cell growth and tumorigenesis (*B*). High levels of genetic instability, caused by chemotherapeutic agents such as cisplatin or missegregation of large numbers of chromosomes (10–15) per division, result in cell death and tumor suppression (*C*).

of the mitotic spindle (29). In all known examples, CENP-E is accumulated during late G_2 and quantitatively degraded at the end of mitosis (30), making it unlikely that reduction in CENP-E would cause defects other than chromosome missegregation and aneuploidy. Consistently, CENP-E is undetectable in nondividing tissues and before late G_2 in cycling cells. Further investigation revealed that *Cenp-E* heterozygous cells do not have elevated levels of DNA damage, have an intact DNA damage response, do not exhibit chromosomal rearrangements, and express wild-type p53 (31).

Examination of animals with half the normal level of CENP-E revealed, as Boveri had predicted, an increased incidence of lymphomas of the spleen and adenomas of the lung. Interestingly, these tumors occurred late in life (19–21 months) with incomplete penetrance (10%). Although this penetrance is lower than had been predicted by some proponents of the aneuploidy hypothesis, it should be noted that it is similar to the percentage of smokers that develop lung cancer (32). More surprisingly, aneuploidy due to *Cenp-E* heterozygosity resulted in a decreased incidence of spontaneous liver tumors, tumors induced with the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA), and tumors caused by homozygous loss of the *p19/ARF* tumor suppressor. Thus, aneuploidy was found to act either oncogenically or as a tumor suppressor depending on the cell type and the presence or absence of additional genetic damage (31).

Discussion: Aneuploidy as a Wild Card

These results have several implications. First, because *Cenp-E* heterozygous cells do not show an increase in tetraploidy, chromosome missegregation per se does not cause cytokinesis failure, as has been suggested (33). More importantly, aneuploidy resulting from chromosomal instability drives an increase in both benign and cancerous tumors, indicating that it is clearly not inconsequential. The long latency and incomplete penetrance of these tumors suggests that only a small subset of the large number of possible abnormal combinations of chromosomes is capable of inducing transformation. It also suggests that the chromosomal complements capable of transformation are more complex than gain or loss of one or a few chromosomes and require multiple generations of segregational errors to evolve.

One possibility is that aneuploidy drives tumorigenesis via loss of the remaining wild-type allele of a tumor suppressor gene after spontaneous mutation of the first allele. However, this is unlikely because aneuploidy due to *Cenp-E* heterozygosity actually delayed tumor onset in mice lacking the p19/ARF tumor suppressor. Additionally, aneuploidy inhibited tumor development in mice after treatment with the mutagenic carcinogen DMBA (31). Thus, the data are more consistent with the hypothesis that misregulated gene expression due to abnormal combinations of chromosomes is driving tumorigenesis in *Cenp-E* heterozygous mice, rather than mutations in tumor suppressors.

The most surprising finding of this study was the identification of a previously unsuspected role for aneuploidy in suppressing tumors. Boveri reported that massive missegregation of chromosomes due to supernumery spindle poles resulted in cell death in sea urchin embryos (3). More recently, this finding has been extended to human cancer cells that missegregate large numbers of chromosomes (10–15 per division) due to complete inactivation of the mitotic checkpoint (34, 35). All three contexts in which *Cenp-E* heterozygosity suppressed tumors have now been shown to contain a preexisting level of aneuploidy that is increased by reduction in CENP-E (29).[1] First, 40% of wild-type liver cells exhibit abnormal anaphase figures consistent with chromosome missegregation (lagging or pole-associated chromosomes) and this increases to 95% after excision of a conditional CENP-E allele (29). Second, $p19/ARF^{-/-}$, $Cenp\text{-}E^{+/+}$ murine embryonic fibroblasts (MEF) exhibit higher levels of aneuploidy than wild-type MEFs but lower levels than $p19/ARF^{-/-}$, $Cenp\text{-}E^{+/-}$ MEFs. Finally, treatment with DMBA causes an increased level of aneuploidy in wild-type MEFs, but *Cenp-E* heterozygous MEFs treated with DMBA exhibit higher aneuploidy still.[1] This suggests a model in which the effects of aneuploidy are similar to those of DNA damage, as proposed by Loeb's "mutator hypothesis" (36). Low levels of instability, caused by mutations in mismatch repair genes or missegregation of small numbers of chromosomes, promote cell growth and tumorigenesis. However, high levels of genetic instability, caused by chemotherapy drugs such as cisplatin or very high rates of chromosome missegregation, lead to cell death and tumor regression (Fig. 1). For aneuploidy, experiments to delineate precisely in what contexts aneuploidy acts oncogenically and those in which it acts as a tumor suppressor are now central to defining how chromosome gain and loss contribute to tumor initiation and progression.

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