Mutations and aneuploidy: Co-conspirators in cancer?

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The role of intragenic point mutations in human cancer is well established. However, the contribution of massive genomic changes collectively known as aneuploidy is less certain. Recent experimental work suggests that aneuploidy is required for sporadic carcinogenesis in mice and that it may collaborate with intragenic mutations during tumorigenesis. The genomic plasticity afforded by aneuploidy could facilitate emergence of protumorigenic gene dosage changes and accelerate accumulation of oncogenes and loss of tumor suppressor genes. These new findings force us to rethink the pathogenesis of carcinoma in ways that have significant implications for diagnosis and therapy.

Introduction

Growing evidence indicates that carcinomas, the most common and lethal of human tumors, may be mechanistically more complex than originally thought. Although it is well established that most carcinomas arise from the stepwise accumulation of genetic changes (Hahn and Weinberg, 2002), the nature and temporal sequence of such changes is less clear. Cancers in general, and carcinomas in particular, exhibit extensive modifications in genome composition, ranging from subtle point mutations to dramatic gains and losses of genetic material (aneuploidy). Though the role of mutations in tumorigenesis has gained strength, ever since Hermann J. Muller proposed that multiple intragenic mutations can cause cancer (Muller, 1927), the contribution of aneuploidy is less certain and hotly debated (Marx, 2002). This debate has reignited an old controversy concerning the dominance of mutations or aneuploidy as the cause of cancer, and has polarized the research community into espousing either one or the other pathway as being both necessary and sufficient for cancer development (Hahn and Weinberg, 2002; Li et al., 2000; Marx, 2002). Here we review recent experimental evidence that argues strongly for collaboration between intragenic mutations (referred to as mutations from this point on) and aneuploidy in the pathogenesis of carcinoma.

Mutations and cancer

Gatekeeper genes that control cell growth and death and caretaker genes that maintain genome integrity are the most common genes implicated in cancer (Kinzler and Vogelstein, 1997). Members of either class can act as oncogenes (OG) when activated by gain of function mutations (e.g., ras, Flt-3, c-kit) or tumor suppressor genes (TSG) when inactivated by loss of function mutations (e.g., p53, Rb, APC) (Weinberg, 1994).

Intragenic mutations in cancer accrue in a small subset of genes

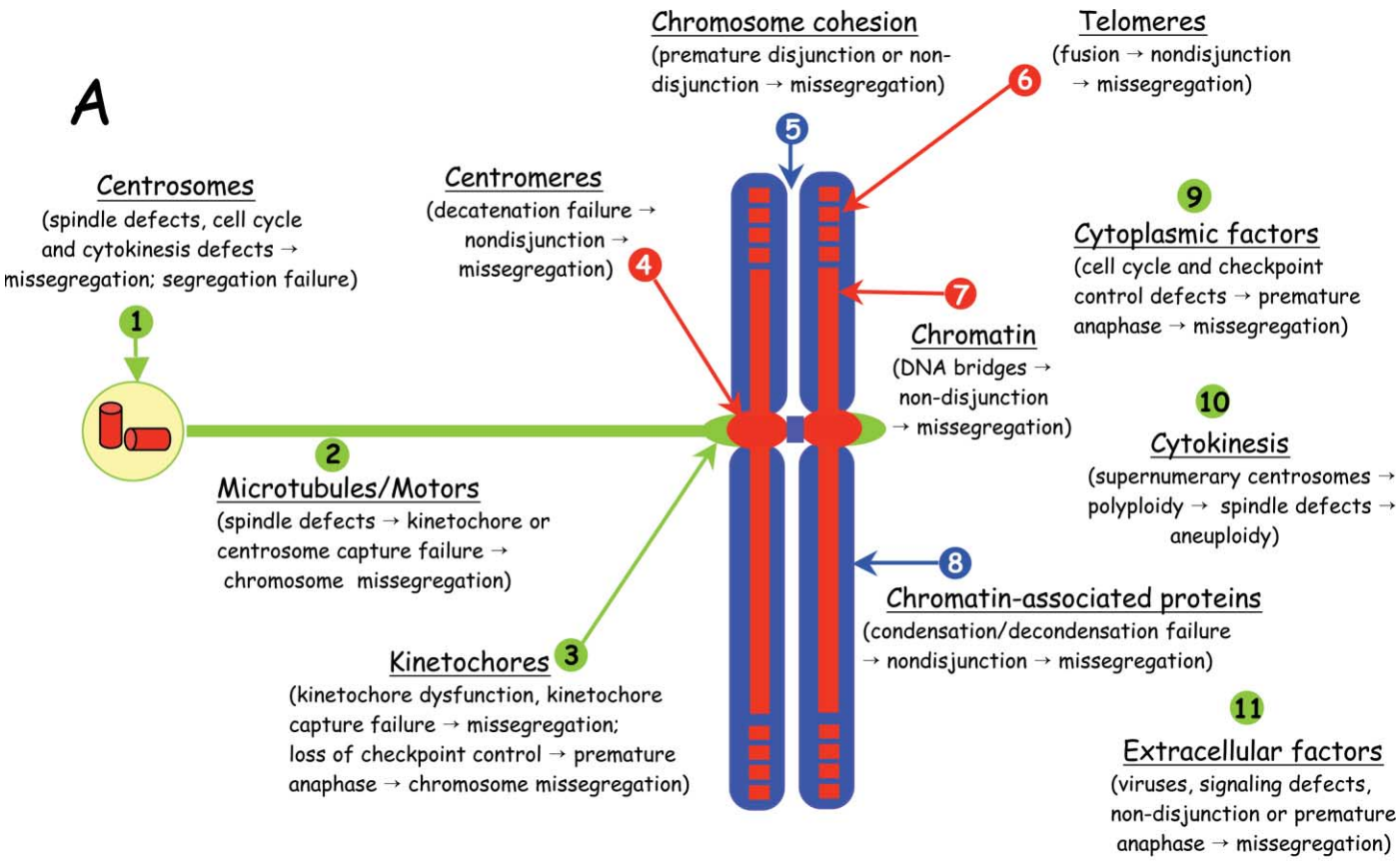
Lawrence Loeb originally postulated that tumor development required cells to acquire a high mutation rate (Loeb, 1991). However, more than thirty years of research has failed to provide compelling support for such a "cancer mutator phenotype" (Marx, 2002), with a few remarkable exceptions (Modrich and Lahue, 1996). Recently, direct measurements of mutations in sporadic colon carcinoma showed that the total number of mutations was considerably lower than predicted by Loeb's model (Wang et al., 2002) and suggested that increased gene mutation rate alone may not be the key to cancer. Moreover, mutations that occur during development of most sporadic human carcinomas are restricted to a small subset of genes, including components of the ras (Sahai and Marshall, 2002), p53 (Levine et al., 1991), Rb (Sherr and McCormick, 2002), Akt (Vivanco and Sawyers, 2002), and Wnt (Taipale and Beachy, 2001) signal transduction pathways (Hunter, 1997).

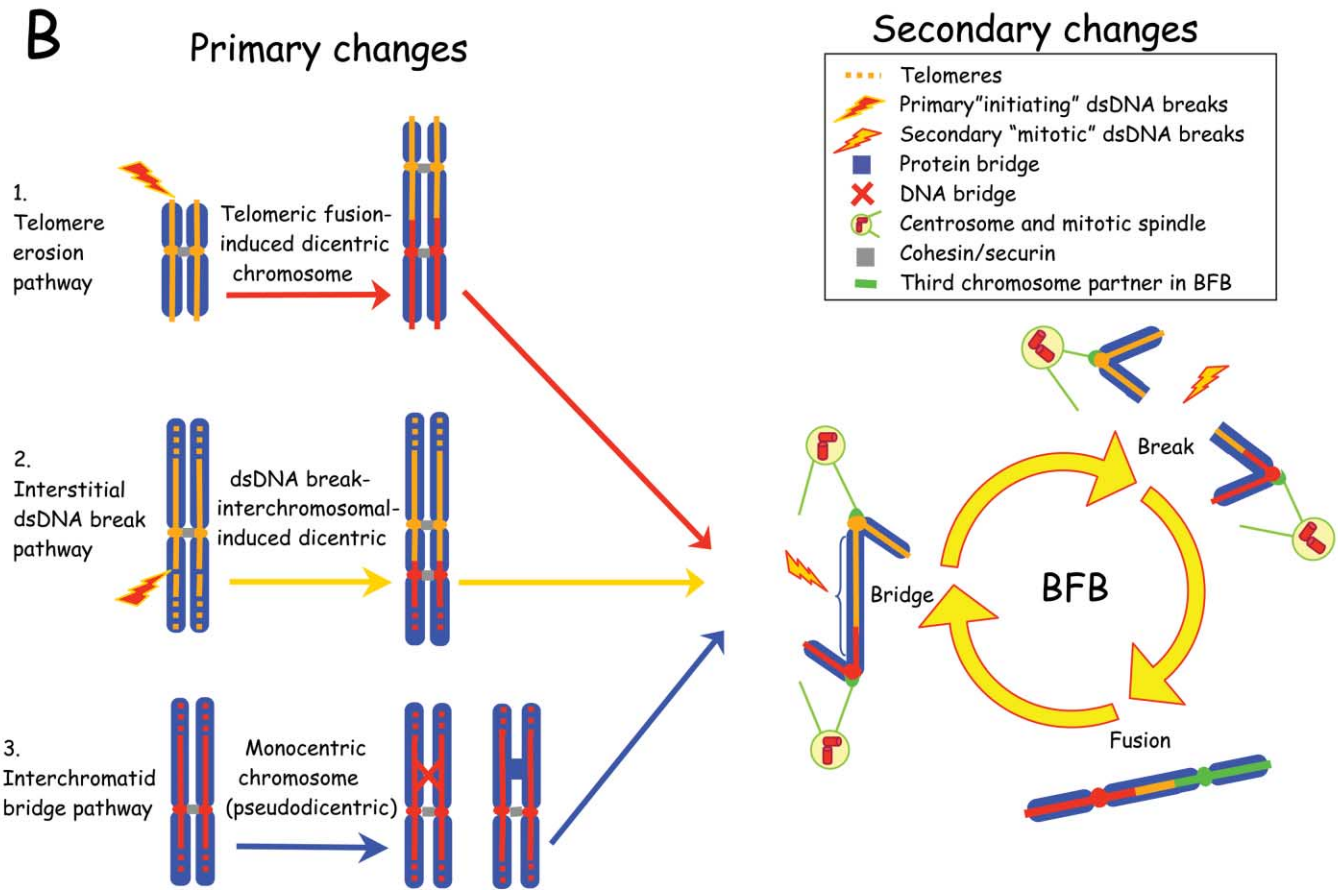
Role of cell proliferation in cancer gene mutation accrual

If the rate of point mutation in cancer is not markedly increased, how can mutations arise in such a restricted set of genes at apparently high rates? The most likely mechanism is that prevalent cancer point mutations confer significant proliferative and/or survival advantages, allowing cells carrying them to quickly become the dominant cell population within a tissue (Tomlinson et al., 1996). Viewed in this context, rapid accrual of mutations in cancer genes driven primarily by increased cell proliferation becomes plausible even in the absence of an increased frequency of total mutations. Selective accrual of mutations could also occur as a result of chemical or physical mutagenesis, provided the mutation rate is low, thus enabling cell proliferation to exert a dominant effect on mutational accrual (Tomlinson et al., 1996). Given the potential of cell proliferation to promote accrual of mutations and tumorigenesis, it is not surprising that multicellular organisms have evolved robust mechanisms to set limits to proliferation potential in somatic cells. The most important of these checkpoint mechanisms are cell cycle arrest and apoptosis triggered by telomere erosion (Chin et al., 1999). In fact, telomere integrity appears to be a critical element in cancer development (see below).

Aneuploidy and CIN in cancer

The majority of carcinomas exhibit unstable aneuploidy. Cells within the same tumor characteristically bear striking differences in chromosome number that appear to arise from continuous losses and gains of chromosomes during mitosis (Figure 1) (Jallepalli and Lengauer, 2001). Tumor cells also exhibit ongoing and apparently stochastic large-scale structural changes such as nonreciprocal translocations, inversions, deletions, insertions, and other types of transpositions of chromosome material. These prevalent numerical and structural forms of genome plasticity, hereafter referred to collectively as chromosome instability (CIN), occur continuously and lead to extensive "genome scrambling." In contrast to carcinomas, leukemias and lymphomas do not exhibit extensive CIN (Heim and Mitelman, 1995). They are often diploid or near diploid and


A
Chromosome cohesion
(premature disjunction or non-disjunction → missegregation)
Telomeres
(fusion → nondisjunction → missegregation)
Centrosomes
(spindle defects, cell cycle and cytokinesis defects → missegregation; segregation failure)
Centromeres
(decatenation failure → nondisjunction → missegregation)
Cytoplasmic factors
(cell cycle and checkpoint control defects → premature anaphase → missegregation)
Chromatin
(DNA bridges → non-disjunction → missegregation)
Cytokinesis
(supernumerary centrosomes → polyploidy → spindle defects → aneuploidy)
Microtubules/Motors
(spindle defects → kinetochore or centrosome capture failure → chromosome missegregation)
Chromatin-associated proteins
(condensation/decondensation failure → nondisjunction → missegregation)
Kinetochores
(kinetochore dysfunction, kinetochore capture failure → missegregation; loss of checkpoint control → premature anaphase → chromosome missegregation)
Extracellular factors
(viruses, signaling defects, non-disjunction or premature anaphase → missegregation)


B
Primary changes
Secondary changes
Telomeres
Primary"initiating" dsDNA breaks
Secondary "mitotic" dsDNA breaks
Protein bridge
DNA bridge
Centrosome and mitotic spindle
Cohesin/securin
Third chromosome partner in BFB
1. Telomere erosion pathway
Telomeric fusion-induced dicentric chromosome
2. Interstitial dsDNA break pathway
dsDNA break-interchromosomal-induced dicentric
3. Interchromatid bridge pathway
Monocentric chromosome (pseudodicentric)
Break
Bridge
BFB
Fusion

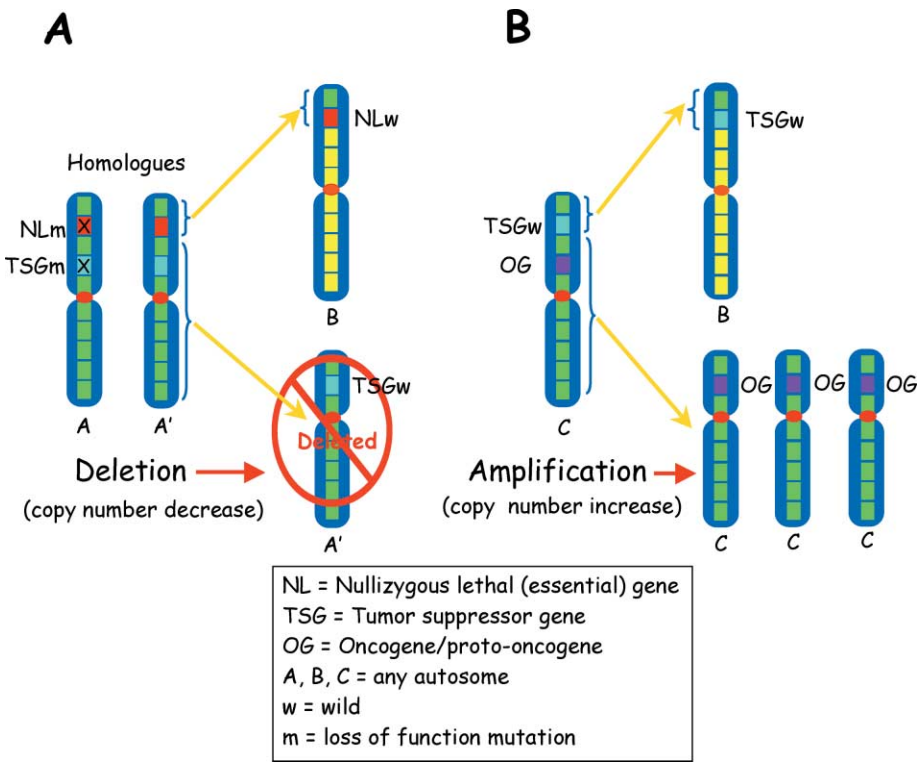


Figure 2. Enabling effect of structural chromosome instability on gene gains and losses

A: Transposition of chromosome material facilitates loss of a TSG.
B: Transposition of chromosomal material facilitates gain of OGs or other growth-promoting genes. See text for more details.

when aneuploid, exhibit relatively stable chromosome complements. This is probably because aneuploidy in lymphoma and leukemia more likely arises as a rare event in a tumor founder cell. What then could be the pathogenic role of CIN in human carcinoma?

Formulated by Boveri a century ago, the so called "aneuploidy theory" of cancer origin has fallen out of favor. This is a consequence of our increased understanding of the molecular biology of cancer, persuasive literature supporting the idea that gene mutation drives tumorigenesis (Balmain, 2001), and the conceptual influence of the identification of a handful of frequently mutated genes in cancer (notably ras, p53, Rb, AKT, PTEN, p16INK4a, and Wnt). However, our current concept of cancer has overlooked the central idea in Boveri's proposal. In essence, Boveri proposed that cancer arose by gains and/or loses of growth-promoting and growth-restraining genes, respectively. He pointed out that rare abnormal mitoses could lead to the "right combination of chromatin determinants" which could unleash cancer (Balmain, 2001). At a time when the concept of "gene" was still being developed in his laboratory (Balzer, 1967), Boveri was the first to propose that acquisition of the appropriate combination of genes ("chromatin determinants") (Boveri, 1914), now known as gene dosage effects, played a role in cancer. Below, we discuss recent data that supports Boveri's original ideas.

Do CIN-enabled gene dosage changes contribute to cancer?

Gene dosage can influence gene expression (Fodde and Smits, 2002). For most genes, with the exception of haploinsufficient genes, loss or mutation of both copies is required before noticeable changes in expression occur. Copy number gains, on the other hand, frequently lead to increased gene expression. Classic examples in cancer are highly amplified genome regions known as homogeneously staining regions and double minute chromosomes. These regions invariably contain genes known to confer cancer phenotypic advantage (e.g., proliferation, resistance to therapy) (Schwab, 1999). While local gene amplification is undoubtedly the driving tumorigenic force in some tumors, most carcinomas do not carry such high-level gene amplifications. Observations over the past ten years strongly suggest that a more subtle yet more pervasive form of gene dosage change may operate in the vast majority of carcinomas (Knuutila et al., 1998, 1999)

Complicity between structural and numerical CIN in the Darwinian emergence of defined protumorigenic gene dosage patterns among related carcinomas

Measurements of relative gene copy number by comparative genomic hybridization (CGH) have shown that subsets of related tumors show similar but not identical patterns of large-scale gene losses and gains throughout the genome (Knuutila et al., 1998, 1999). Remarkably, the same tumors show extensive chromosome fragment transpositions (chromosome scrambling) when mapped by spectral karyotyping (SKY) (Schrock et al., 1996), but they do not share the same (nonrandom) structural chromosome changes (Figure 2). How could shared gene dosage changes be reconciled with a multitude of independent chromosome structural changes?

Changes in gene dosage may be modulated by the presence of adjacent genes on the same chromosome with antagonistic activities (e.g., growth promotion and inhibition), a condition akin to classic linkage disequilibrium (nonindependent assortment of genes during cell division). In tumors with inactivating mutations of a TSG allele, deletion of the normal allele is required for expression of the TSG phenotype

Figure 1. Pathways leading to numerical and structural CIN

A: Numerical CIN.
B: Structural CIN.
See text for more details.

(Knudson, 2001). This often occurs in tumors by mitotic deletion of large chromosome fragments (Figure 1) (Thiagalingam et al., 2001). However, deletion of the chromosome fragment containing the normal TSG allele may be encumbered by the presence of the only remaining normal copy of a nullizygous lethal gene in its vicinity (telomeric, Figure 2). This situation could arise often, since every chromosome arm contains hundreds of nullizygous lethal genes, and any of these could be inactivated in the chromosome arm containing the mutated TSG. Under these conditions, deletion of the normal tumor suppressor allele by loss of the chromosome arm could occur only if the normal nullizygous lethal gene is relocated to another chromosome before arm deletion (Figure 2). This mechanism could also operate when LOH is the primary event and deletional loss of the other copy of a TSG is the secondary event (Frei, 1993). In an analogous manner, accumulation of genes that promote tumor growth (OG or wild-type allele) may be opposed by strong growth inhibitory genes in linkage disequilibrium. This opposition could be overcome if the OG and TSG are first dissociated from each other during a CIN event and placed into independently segregating chromosomes at mitoses (no longer in linkage disequilibrium).

Constraints to gene losses and gains by linkage disequilibrium suggest a tantalizing model for gene dosage changes in cancer. The extensive genome scrambling typical of all carcinomas may be nothing more than evolution toward a state of "linkage equilibrium" of cancer genes enabled by CIN and driven by Darwinian selection. Chromosome transfer experiments in cancer cells support this view (Imreh et al., 1997). Thus, the induction of gene dosage changes may be central to the role of CIN in cancer development. Although it is generally acknowledged that extensive genome scrambling is the norm in carcinoma, its cause(s) are less generally agreed upon. Recent modeling of carcinoma in mice strongly suggests a dominant mechanism.

Double-strand DNA (dsDNA) breaks and the origins of scrambled aneuploid genomes

While point mutations arise by misrepair of DNA base damage or misincorporated DNA bases (Friedberg, 2001), the genome scrambling typical of carcinomas is most likely catalyzed by inappropriately repaired dsDNA breaks (Sharpless et al., 2001) or by eroded telomere ends that are sensed and processed by cells as dsDNA breaks (Saretzki et al., 1999). Evidence that supports this idea comes from both dsDNA break repair deficiency syndromes and experimental data in mice with knockout caretaker genes involved in homologous and nonhomologous DNA end-joining DNA repair (Maser and DePinho, 2003; Mills et al., 2003; van Gent et al., 2001).

Unrepaired and unprotected dsDNA breaks, including exposed telomere ends, are fusogenic and tend to be repaired by joining to other dsDNA ends. The importance of properly repaired dsDNA breaks is suggested by the increased incidence of tumors in humans with deficiencies of homologous recombination or nonhomologous end joining repair, or with defects in the signaling pathways that sense unrepaired dsDNA breaks such as in ataxia telangectasia (Meyn, 1997; Shiloh, 2003; Thompson and Schild, 2002). dsDNA breaks or eroded telomeres repaired to heterologous ends may generate dicentric chromosomes or ring chromosomes that initiate breakage-fusion-bridge (BFB) cycles (see below, Figure 1). This can lead to extensive genome remodeling and scrambling characteristic of carcinoma and cause nonreciprocal translocations, the most common structural chromosome abnormalities in carcinoma (Gisselsson, 2003; Lengauer, 2001). Whether BFB cycles generate defined or random breaks that contribute to cancer is still an open question. One possibility is that breakpoints are random but there is an overrepresentation of those that bring TSG and OG or TSG and nullizygous lethal genes into linkage equilibrium (by relocation to different chromosomes and independent sorting during mitosis) because they have a selective advantage. Moreover, the apparent randomness of structural changes in carcinomas with similar gene dosage patterns may not reflect random positions of breakpoints that generated the transposing fragment. Rather, it may result from random transposition of the chromosome fragment into any chromosome, as well as the random position of the breakpoint in the chromosome that accepts the transposing fragment. In this manner, related initiating events might lead to a variety of apparently unrelated structural changes.

A milestone in experimental human cancer modeling has been recently achieved by the elegant studies of DePinho and colleagues (Artandi et al., 2000). Prior to these studies, most murine cancer models (knockouts, knockins, transgenics) reproduced some aspects of human lymphomas and sarcomas, but did not reproduce sporadic human carcinomas well. DePinho and collaborators, using late-generation telomerase-null mice, followed emergence of carcinomas with a remarkable resemblance to human carcinoma. These murine carcinomas were aneuploid and exhibited extensive CIN, including gains and losses of chromosomes characteristic of human carcinoma. Remarkably, the chromosome regions gained or lost were syntenic with those gained or lost in orthologous human carcinomas (O'Hagan et al., 2002). These findings provide the strongest experimental evidence, albeit indirect, for defined (recurrent) abnormal gene dosage patterns in carcinoma development. Moreover, epithelial carcinogenesis in telomerase-null mice was dependent on defective p53 function (Chang et al., 2001), raising the possibility that the critical role of some checkpoint gene mutations (e.g., p53) is to permit emergence of protumorigenic gene dosage changes. This observation also highlights the nearly certain interdependence of CIN and critical gene mutations, since loss of p53 function is permissive for CIN and gene dosage changes.

The role of mitosis in chromosome breakage and missegregtion

Ever since their visualization by von Hansemann more than 100 years ago (von Hansemann, 1890), pathologists have sought atypical mitoses as hallmarks of malignancy in histological sections of tumors, yet they rarely pause to consider the implications of such findings. Cancer mitoses may in fact provide two key contributions to carcinogenesis. They perpetuate random break cycles during BFB cycles, leading to extensive genome scrambling that enables emergence of defined gene dosage changes. They also induce chromosome missegregation at a high frequency, thus inducing CIN and gene dosage change patterns (Figure 1). While BFB cycles appear to be the dominant pathway to structural chromosome changes, it is unclear what the initiating dsDNA break may be (Figure 2). Clearly, in telomerase-deficient mice, critically shortened telomeres are the "dsDNA break" and suggest a very attractive model for human carcinoma. In human carcinoma, however, this is less well established, and interstitial DNA breaks may play a significant role as well (Difilippantonio et al., 2002). Moreover, while the mechanism of repair of dsDNA breaks is well understood (Hoeijmakers, 2001), its contribution to structural chromosome changes is currently poorly understood. Finally, though the

nearly universal occurrence of chromosome missegregation in carcinomas is well established, the dominant mechanisms leading to it remain poorly defined. Potential contributors include centrosome dysfunction, anaphase checkpoint malfunction, and cytokinesis failure (Figure 1) (Jallepalli and Lengauer, 2001; Pihan and Doxsey, 1999). Future studies will be required to determine whether gene dosage changes alone can reproduce phenotypic characteristics of carcinomas. This may pose some difficulties, since large-scale gene dosage changes are poorly tolerated by normal cells and invariably lead to checkpoint activation and cell cycle arrest (Andreassen et al., 2001).

In conclusion, recent results in murine carcinoma models suggest that expression of the cancer phenotype in carcinomas invariably involves gene dosage changes as well as intragenic mutations. Most gene dosage changes occur via chromosome transpositions catalyzed by chromosome BFB cycles coupled to mitotic chromosome missegregation. At least some mutations are critically permissive for gene dosage changes. The genome plasticity generated by these mechanisms may facilitate not only early tumor development but also tumor evolution toward enhanced growth, loss of normal apoptotic mechanisms, and resistance to therapy.

Acknowledgments

Our work is supported by NIH GM51994 (S.J.D.) and Department of Defense PC970425 (S.J.D., G.P.)

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