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CIN-ful cancers

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Abstract Aneuploidy has long been recognized to be a cardinal feature of many neoplasias. However, the role of aneuploidy in tumorigenesis continues to be a matter of debate. We believe that aneuploidy in cancers is the result of chromosomal instability, a process in which dividing cancer cells segregate their chromosomes with decreased fidelity. Here we discuss our definition of chromosomal instability, evidence for its causal role in tumor development, and suggestions regarding the mechanisms that initiate chromosomal instability in cancer cells.

Keywords Aneuploidy · Chromosomal instability · Cancer

Microsatellite instability

Our attempts to uncover the mechanism underlying aneuploidy began with our analysis of the smaller fraction of colorectal cancers that are not aneuploid. Approximately 15% of colorectal cancers exhibit a form of genetic instability that is characterized by mismatch repair (MMR) deficiency, generally due to inactivation of the hMLH1 or hMSH2 MMR genes. Loss of MMR function renders tumor cells susceptible to the acquisition of somatic mutations throughout the genome. Simple repeat sequences are particularly susceptible to mutations in the absence of MMR, and the instability in these tumors is thereby often referred to as microsatellite instability (MIN) [9, 13, 15]. Cancer cells that possess MIN have a diploid or near-diploid chromosomal content and have a mutation rate at the nucleotide level that is two to three orders of magnitude greater than that observed in normal cells [1].

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Chromosomal instability

The remaining 85% of colorectal cancers, and an even larger proportion of other solid tumor types, do not exhibit MIN. However, they do contain an abnormal chromosomal content—that is, they are aneuploid. We hypothesized that aneuploid cancers may have an analogous genetic instability to the MMR deficiency found in diploid cancers. Importantly, aneuploid cancers do not have an accelerated rate of point mutation, but they do have abnormal karyotypes. To understand whether the abnormal karyotype was the silhouette of an inherent instability process, the rate at which colon cancer cell lines gain and lose chromosomes was measured [7]. Clones were generated and expanded through a defined number of generations before they were examined by fluorescence in situ hybridization with centromeric probes. In cell lines that did not exhibit MIN, the probability of losing or gaining a chromosome was approximately 0.01 per chromosome per cell division. Importantly, the corresponding rate in MIN cell lines was much lower and could not be accurately determined. The accelerated rate of chromosomal gains and losses was termed chromosomal instability (CIN). CIN, like MIN, was postulated to allow cells to rapidly acquire genetic changes required for tumorigenesis.

Defining CIN

It is important to recognize that CIN refers to the rate with which whole chromosomes or large portions

thereof are gained or lost in cancers. It is not synonymous with the state of aneuploidy that is observed in a static image of a cancer cell's chromosomal content [8]. In other words, while CIN is a process that drives most cancers to aneuploidy, aneuploidy per se does not imply the existence of CIN. On the other hand, there are several ways in which a cancer cell could become aneuploid in the absence of CIN. First, the cell could have divided many more times than normal cells within a tissue, without any difference in the rate of chromosomal change per division. It is known that gross chromosomal changes occur in normal cells, so this possibility is a real one. Second, exposure to endogenous or exogenous agents could potentially induce aneuploidy, perhaps in the same way as pharmacologic agents that disrupt spindle formation. The resultant daughter cells would be aneuploid, but not chromosomally unstable, in future generations.

It is also possible that cancer cells develop chromosomal changes at the same rate as normal cells, but that gross chromosomal changes are only lethal to normal cells. This possibility is bolstered by the knowledge that mutations in cancer genes disrupt the capacity for cells to undergo apoptosis. Though the ability to survive chromosomal changes might be scored as CIN in some assays, the mechanisms underlying this process would be very different. For example, mutations in genes that control the G_2 checkpoint might result in CIN by stimulating chromosomal changes, whereas mutations in genes that control apoptosis would have no effect on the rate at which such changes occur if one measured the rate in total cells rather than simply measuring it in surviving cells.

Though we define CIN as an increased rate at which whole chromosomes or large portions thereof are gained or lost in cancers, it is important to point out that CIN has only been formally demonstrated for whole chromosome losses [7]. At present, there is no assay that can reliably measure the rate of formation of other gross chromosomal changes. These include those changes that are observable at the cytogenetic level, such as rearrangements, deletions, insertions, inversions, and amplifications, as well as more subtle changes including unequal sister chromatid exchange and gene conversion. Such sub-chromosomal changes are at least as common as losses or gains of whole chromosomes. Whether such changes reflect an underlying CIN rather than one of the other mechanisms noted above is an essential question for future research.

The cause of CIN

Given the pervasiveness of aneuploidy in cancers and our limited knowledge of its origins, it is not surprising that numerous ideas have been invoked to explain it. One of the earliest explanations came following the observation that most late-stage cancer cells contain between 60 and 90 chromosomes—many are near-triploid in chromosome number [4]. This near-triploid state could be due to tetraploidization and subsequent chromosome loss. There are a number of ways in which cells can become tetraploid, and chromosomes can be lost either in aggregate or individually. It is possible in this scenario that the chromosomal complement following a tetraploidization event is inherently unstable and that CIN is a trivial consequence of the near doubling of the genetic content.

Two lines of evidence make this hypothesis unlikely. First, the CIN phenotype appears to be dominant, as it can be conferred upon a chromosomally stable, diploid cell when it is fused with a CIN cell. These experiments indicate that the mere presence of an abnormal number of chromosomes is not sufficient to lead to CIN, as the fusion of two non-CIN cells, resulting in tetraploidization, does not result in CIN [7]. Second, a small fraction of cancers contain mutations or abnormal expression patterns of spindle checkpoint genes such as hBub1 and hMad1 [2, 10, 12]. Recreation of these mutations in non-CIN lines can convert them to CIN, with resultant aneuploidy. Such results suggest that there may be a genetic mechanism underlying CIN in at least a subset of cases.

Many other genetic mechanisms that could potentially lead to CIN have been suggested and discussed [3, 6] (Fig. 1). Furthermore, there can be no guarantee that the basis of CIN is genetic. Epigenetic events that do not involve mutational changes in nucleotides could certainly play a major role. It is also possible that CIN is related to the abnormal architectural features of the cancer cell, secondarily resulting in changes in spindle structure. Indeed, many reports of abnormal centrosomes and spindles in various types of cancer have been identified [11]. Definitive evidence that such changes are the cause rather than the consequence of CIN will, however, require a much deeper understanding of the mechanisms that normally safeguard the integrity of the chromosome complement. Identification of the key human genes responsible for CIN, either through abnormal expression or structure, is just in its infancy.

The genetic basis of CIN

The genetics of CIN remain a mysterious and somewhat controversial issue in cancer research. At one extreme, some investigators have argued that CIN is self-propagating and does not require specific genetic mutations [3]. On the other hand, just as mutations in mismatch repair genes were initially found to cause MIN in bacteria and yeast before their discovery in human cancers, numerous genes (more than 100) have been found to cause chromosomal instability in yeast [14]. These include genes involved in mitotic spindle assembly and dynamics, chromosome metabolism, cell-cycle regulation, and checkpoint control. Most of these 100 genes have several homologues in the human

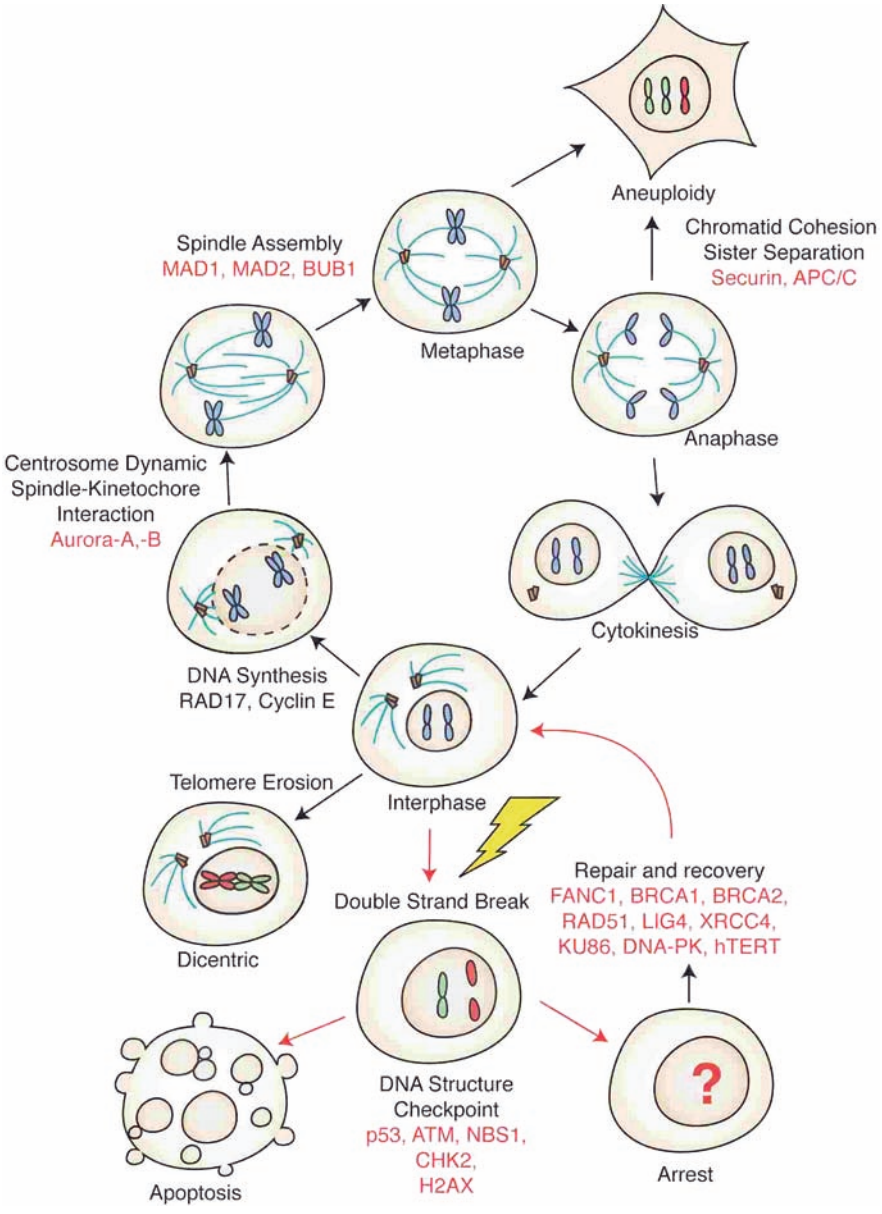

Fig. 1 Pathways to aneuploidy. The figure depicts the phases of a cell cycle and the potential mechanisms through which aneuploidy can arise in cell division. The *upper circle* outlines the dynamic activity of DNA during a cell cycle. The genes that may have altered activity in cancers and which could conceivably play a role in aneuploidy are indicated in *red*. The most likely proximate cause of aneuploidy in cancers is chromosome missegregation during mitosis, as a result of problems in spindle-kinetochore attachments, spindle assembly, chromatid cohesion, or sister separation. While these mitotic events may be the only cause of aneuploidy, it is also conceivable that disruptive events during interphase can produce mitotic errors. These distal events could be related to errors in DNA synthesis or assaults on the genome by telomere erosion or double strand breaks. Telomere erosion can produce dicentric chromosomes, and consequently lead to missegregation during the cell's next mitosis. Double strand breaks, on the other hand, induce cell cycle checkpoints which force the cell to undergo either apoptosis or arrest while repair mechanisms attempt to restore genomic integrity (reprinted with permission of AAAS from "Debate Surges Over the Origins of Genomic Defects in Cancer" by Jean Marx. Science 2002;297:544–546. Copyright 2002 AAAS)

genome, and research into their contributions to cancer is just beginning to achieve fruition. There should also be a clear difference between the genes that are required for the maintenance of genetic stability in normally dividing cells ("genomic integrity" genes) and genes that are genetically altered in cancers, and thus causally contribute to chromosomal instability ("cancer CIN" genes). A great deal of confusion in the field of chromosomal instability, and much of the controversy, arises from the failure to draw this important distinction.

Conclusion

Epidemiologic data initially suggested that cancers undergo four to six rate-limiting bottlenecks in the course of their development [5]. Since this hypothesis, many researchers have argued whether the normal rate of mutation in dividing cells is sufficient to account for the high rate of incidence of cancers within the general population. The discovery of mutations in DNA repair enzymes (MMR mutations in MIN cancers and nucleotide-excision repair gene mutations in xeroderma pigmentosum) lent tremendous evidence to the argument that cancer cells must undergo specific changes that allow an accelerated mutation rate. The accelerated rate of gains and losses of chromosomes in CIN seems to provide a parsimonious explanation for the aneuploidy characteristic of most solid tumors. Whether tumorigenesis selects for CIN, as it would for mutations in tumor suppressors and oncogenes, and whether this CIN is a consequence of specific genetic mutations, are important areas for future research.

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