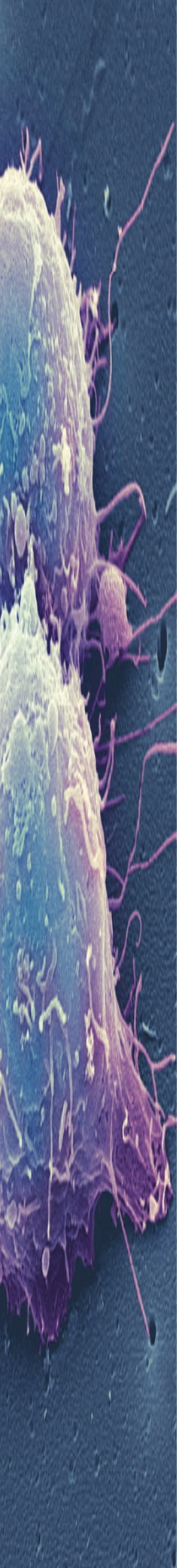

Aneuploidy and cancer

Harith Rajagopalan & Christoph Lengauer

The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, 1650 Orleans Street, Baltimore, Maryland 21231, USA (e-mail: lengauer@jhmi.edu)

In contrast to normal cells, aneuploidy — alterations in the number of chromosomes — is consistently observed in virtually all cancers. A growing body of evidence suggests that aneuploidy is often caused by a particular type of genetic instability, called chromosomal instability, which may reflect defects in mitotic segregation in cancer cells. A better understanding of the molecular mechanisms leading to aneuploidy holds promise for the development of cancer drugs that target this process.

A fundamental principle underlying our understanding of tumorigenesis is that cancers arise from the sequential acquisition of genetic alterations in specific genes[1,2]. These changes (mutations and amplifications, for instance) occur in individual cells within a population: each change attains fixation from a wave of clonal expansion due to the relative growth advantage that the new mutation confers on the cell. In this way, genetic events represent rate-limiting bottlenecks in the clonal evolution of cancers. But the number of bottlenecks needed for a single cell to develop into a metastastic cancer is not clear. Mathematical extrapolations have suggested that most cancers require six to ten such clonal events to fully mature[3,4].

In this context, let us consider one of the most common properties of cancers — aneuploidy. More than a century ago, one of the first things that researchers noticed when they looked at cancer cells under the microscope was that tumour cells often had excess chromosomes[5,6]. Normal human cells (even those invading and immediately surrounding cancers) contain an invariable complement of 46 chromosomes. However, most cancers contain cells that not only possess an abnormal number of chromosomes (often between 60 and 90) but that also differ from each other in the number of chromosomes they contain. Furthermore, these chromosomes commonly have structural aberrations that are vanishingly rare in normal cells: inversions, deletions, duplications, and translocations (Fig. 1). These numerical and structural abnormalities define aneuploidy.

Why are cancers aneuploid? Because it is so common, it has been suggested that aneuploidy, like defects in signalling pathways, is essential for tumorigenesis[7,8]. Alternatively, aneuploidy may be a meaningless consequence of deregulated growth in cancer cells; simply a cytogenetic silhouette of other processes that are essential for tumorigenesis. In addition to the question of aneuploidy's relevance to tumorigenesis, its genetic basis is also unclear. Although many believe that aneuploidy can be caused by genetic changes within tumours, others have suggested that aneuploidy is entirely independent of gene mutations[7]. To tease apart these possibilities, we return to observations on specific cancers and attempt to draw implications from these studies. We review historical and modern efforts to explain the causes and consequences of aneuploidy and its contribution to cancer. Finally, we consider the therapeutic implications offered by these findings.

Defects in mitosis

Mitotic defects in tumour cells with aneuploidy were first described by David Hansemann more than a century ago[9]. In tissue sections from various carcinomas, he encountered mitotic figures (chromosome architectures) that were abnormal in size and structure. In particular, he described asymmetrical mitoses in cells displaying bipolar anaphase and telophase. Here, the two groups of segregating sister chromatids contained unequal amounts of chromatin. Hansemann also described two additional chromosomal structures that often coincided with asymmetrical mitoses: the formation of anaphase bridges and multipolar mitoses. All three processes can result in abnormalities in chromosome numbers and to a gradual loss of heterozygosity by sequential loss of mitotically unstable chromosomes[10,11].

However, a significant proportion of solid tumours show polyploid chromosome numbers (duplication of a full chromosome complement), which would not be expected to arise from a gradual loss of individual chromosomes from a diploid cell. Studies of the cytogenetic evolution in breast cancer have suggested that a highly aneuploid state could originate from a tetraploidization (whole genome duplication) event concurrent with a gradual loss of individual chromosome copies[12]. This idea is supported by the finding of a high frequency of tetraploid cells in certain preneoplastic lesions, such as Barrett's oesophagus[13] and ulcerative colitis[14]. Further evidence stems from the observation that aneuploid tumours often show a duplication of some of their structurally rearranged chromosomes, including balanced translocations. The precise mechanisms behind such a whole-genome duplication remain to be elucidated. Recent experiments suggest that short telomeres could have a key role, as telomerase-negative immortalized cells tend to develop a tetraploid cell population[15].

When a tumour cell acquires double the number of chromosomes, it also acquires double the number of centrosomes. This raises the question of whether abnormal centrosome numbers in tumours are merely side effects of tetraploidization or whether they have a causal role in tumour evolution. An *in vitro* model overexpressing the kinase Aurora A has shown that tetraploidization is a major route to centrosome amplification[16]. Approximately 80% of invasive breast tumours show abnormalities in the structure and/or number of centrosomes, and abnormalities in centrosome size and centrosome number show a positive correlation with aneuploidy[17]. Centrosomal abnormalities have also been detected at the breast carcinoma *in situ* stage, indicating that they develop early in the neoplastic process[18].

Lessons from cancers

Moving away from a descriptive approach to understanding aneuploidy, more recent work has focused on understanding the teleological basis for why cancers develop this ubiquitous property. Ironically, new insights into aneuploidy have come from the analysis of the small fraction of cancers that are not aneuploid. Approximately 15% of colorectal cancers show a form of genetic instability that is

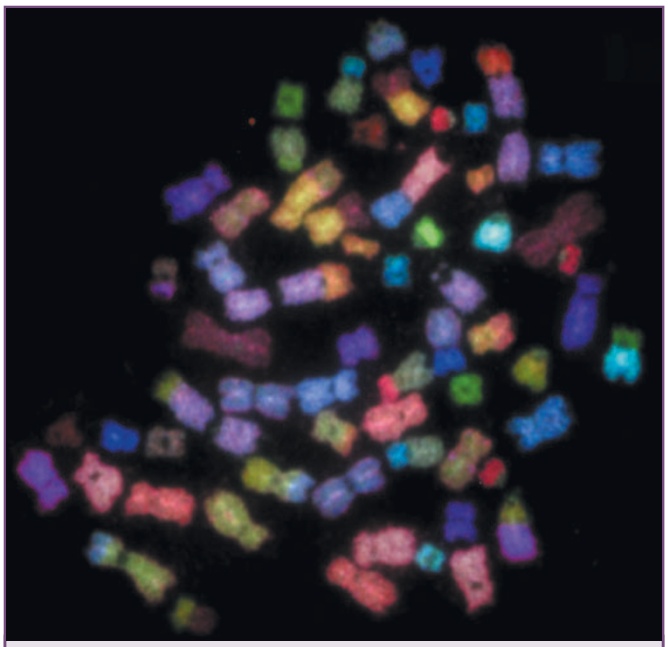

Figure 1 Multicolour-fluorescence *in situ* hybridization of an aneuploid non-small cell lung cancer. Metaphase chromosomes are stained in 23 different colours (provided by M. R. Speicher).

characterized by mismatch repair (MMR) deficiency. This is generally due to inactivation of the MMR genes *hMLH1* or *hMSH2* (ref. 19). Loss of MMR function renders tumour 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 genetic instability in these tumours is often referred to as microsatellite instability (MIN or MSI)[20–22]. Cancer cells that possess MIN have a mutation rate at the nucleotide level that is two to three orders of magnitude greater than that observed in normal cells. However, these cancer cells retain a diploid or near-diploid chromosome content[23].

Another example of cancers without aneuploidy is the hereditary cancer syndrome Xeroderma pigmentosum (XP). XP patients, who inherit a deficiency in one of the genes in the nucleotide excision repair (NER) pathway, develop skin cancers whose genomes are characterized by a high mutation rate at pyrimidine dimers. Unlike sporadic skin cancers (basal cell carcinomas, squamous cell carcinomas, and melanomas) that develop in non-XP patients, NER-deficient skin cancers are not aneuploid[24].

To summarize, although most solid tumours are aneuploid, the few that do not show aneuploidy seem to have inactivated specific DNA-repair pathways. The implication is that these defects allow cancers to accelerate their mutation rates. Indeed, an elevated mutation rate may be necessary to allow cancers to acquire the numerous genetic changes required for tumorigenesis. Given the mutual exclusivity of DNA-repair inactivation and aneuploidy, the corollary argument is that aneuploidy may reflect a different form of genetic instability which accelerates the accumulation of mutations, albeit by different mechanisms.

Aneuploidy and chromosomal instability

One attempt to understand the processes responsible for aneuploidy involved measuring the rate at which colon cancer cell lines gain and lose chromosomes[23]. Clones were generated and expanded through a defined number of generations before they were examined by fluorescence *in situ* hybridization with DNA probes specific for centromeric regions of individual chromosomes. In cell lines that did not show MIN, the probability of losing or gaining a chromosome was 0.01 per chromosome per cell division. The corresponding rate in MIN-cell lines was much lower and could not be accurately determined. This accelerated rate of chromosomal gains and losses that can lead to aneuploidy was termed chromosomal instability (CIN), and has been suggested as an alternative form of genetic instability. What benefit might chromosomal instability confer on a dividing cancer cell? Conceivably, CIN could accelerate the rate of loss of heterozygosity of a tumour suppressor gene and/or effectively amplify an oncogene by duplicating the chromosome on which it lies, thus contributing to tumorigenesis.

Many cancer-cell chromosomes also possess structural abnormalities, including interstitial deletions, inversions and translocations, but it remains unclear whether these changes occur at a higher rate in cancer cells than in normal cells. These structural changes are not measured by the CIN assay described above. It is useful, however, in that it suggests that defective chromosomal segregation leading to CIN may be key to understanding aneuploidy. It should also be noted that the invocation of chromosomal instability as the cause of aneuploidy in cancer is simply one hypothesis.

Molecular mechanisms of chromosomal instability

Assuming that chromosomal instability is one path leading to aneuploidy, the next challenge is to try to understand the mechanisms underlying chromosomal instability. Mounting evidence implicates the mitotic spindle checkpoint as the point of failure in CIN (ref. 25). The normal function of the spindle checkpoint is to ensure that all chromosomes are correctly aligned in metaphase cells and properly attached to the mitotic spindle before chromosome separation can proceed. Chromosomally unstable cells in tissue culture do not arrest in mitosis as well as normal cells when they are incubated with microtubule-disrupting agents. A partially compromised checkpoint may represent a balance that weighs an improved fitness in changing environments against the possibility of apoptosis from numerous genetic insults[26].

Because of the role the spindle checkpoint seems to have in CIN, mutations in putative CIN genes may be identified by examining the components of the kinetochore and spindle checkpoint apparatus (Fig. 2). The ever-expanding list of proteins that have a role in these

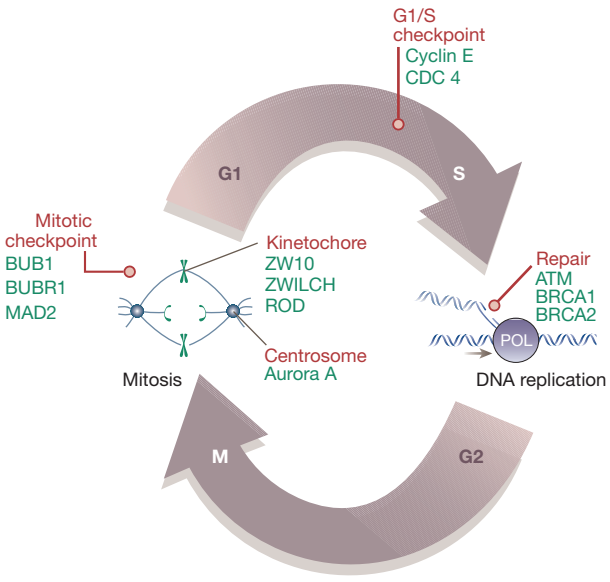

Figure 2 Multiple roads to aneuploidy. The schematic illustrates a simplified cell cycle, highlighting processes that have been implicated in the advent of aneuploidy. Several pathways within the cell cycle (indicated in red) can be disrupted. Genes (indicated in green) associated with these processes and structures have been found to be mutated or functionally altered in aneuploid cancers.

processes have been reviewed elsewhere[27–29], but we shall focus on those that meet one or both of two specific criteria. A candidate can be called a 'CIN gene' only if: (1) there is unambiguous evidence for an alteration in an encoded protein's expression or function in cancers compared to normal cells; and (2) that reconstitution of this alteration confers CIN on a diploid cell in tissue culture[30].

The first example of CIN being caused by mutations in specific genes came from studies of the mitotic-spindle-checkpoint genes *hBUB1* and *hBUBR1* (ref. 25). These genes are mutated infrequently in colorectal cancers and in other tumour types, and mutant alleles of *hBUB1* and reduction of murine BUBR1 expression confer CIN on dividing diploid cells[25,31,32]. Recently, hereditary mutations in *hBUBR1* have been found in individuals with mosaic variegated aneuploidy, a rare disease with increased cancer risk[33]. Another spindle component gene, *hMAD2*, is transcriptionally silenced in breast and other cancers[34]. Genetic inactivation of a single allele of *hMAD2* or overexpression (perhaps as a result of inactivation of the retinoblastoma (Rb) tumour suppressor pathway) confers CIN on diploid cells[35,36]. Mutations in other kinetochore- and centromere-associated genes have also been identified, such as *hZW10*, *hZWILCH*, and *hROD*, but evidence for their role in CIN so far stems purely from their homology to CIN genes in *Drosophila* or yeast[37].

Numerous other proteins function upstream of the kinetochore but are nevertheless integral to chromosome segregation, and may therefore play a role in CIN. In addition to kinetochore components and spindle-checkpoint proteins, the centrosome has been implicated in chromosomal instability. So far, no mutations in any genes encoding centrosome components have been identified in human cancer. Amplification and overexpression of Aurora-A kinase (STK15/BTAK) has been observed in tumours in association with centrosome amplification and aneuploidy[38].

Even further upstream, amplification/overexpression of *cyclin E* and mutation of *hCDC4* (both previously implicated in G1–S phase transitions) have been identified in aneuploid cancers and have also been associated with CIN (refs 39, 40). Intriguingly, cells lacking functional *hCDC4* demonstrate the same inability to arrest in mitosis in response to microtubule-disrupting agents as cells with mutations in kinetochore genes (D. A. Dezentje and S. Kern, personal communication). Therefore, it seems that even mutations and alterations of genes that might be expected to act at points temporally outside the metaphase–anaphase transition can lead to failure of the spindle checkpoint in CIN cancers[26].

In addition, hereditary mutations in recombination and DNA-repair genes (*ATM*, *BRCA1* and *BRCA2*) that have been shown to cause tumour predisposition presumably act by initiating CIN. Mouse knockout experiments have provided functional evidence to support the idea that these genes can contribute to aneuploidy[41,42]. However, whether mutational inactivation of these genes results in increased rates of chromosomal gains/losses has not been elucidated.

It is perhaps not surprising that several routes leading to chromosomal instability, rather than a single mechanism, are now being uncovered in cancer cells. In yeast, mutations in over 100 genes can lead to CIN (refs 43, 44). These genes normally express proteins that have roles in disparate aspects of cellular life and would not otherwise be linked. Ultimately, our understanding of CIN will require the same comprehensive genetic and cell biological analysis of human genes and proteins that has already been accomplished in unicellular organisms. In the past few years we have witnessed an explosion in this sort of phenotypic dissection, and are hopeful that soon we will develop the level of understanding necessary to account for most genetic causes of aneuploidy in all cancers.

Implications for cancer prognosis and therapy

The scientific pursuit of aneuploidy and chromosomal instability is a fascinating biological question, but more significantly, it has the potential for tremendous clinical impact. First, aneuploid and/or chromosomally unstable cancers are likely to have a poorer prognosis than diploid cancers — the degree of aneuploidy correlating with the severity of the disease[45,46]. The reasons for the poorer prognosis is unclear, and somewhat surprising given that mismatch repair deficient cancers would otherwise be expected to have greater genetic fitness than chromosomally unstable cancers[4]. Another important point about prognosis is that ploidy status is generally not used to guide treatment regimes for patients with solid tumours. This is true despite the fact that ploidy seems more predictive of clinical outcome than other commonly used tools[47].

Chromosomal instability may also contribute to a cancer's ability to acquire chemoresistance. Although mechanisms of resistance to common chemotherapeutic agents are largely unknown (see review in this issue by Lowe *et al.*, page 307), a few select cases indicate that chromosomal instability may have an important role. For instance, resistance to Gleevec (a tyrosine kinase inhibitor, also known as STI-571), which is used in the treatment of chronic myelogenous leukaemia (CML) and a few other tumour types, has been shown to arise in CML patients whose cancers have undergone blast crisis[48] (see also introduction in this issue by Sawyers, page 294). Resistance to Gleevec arises from point mutations within the BCR–ABL fusion protein that is the hallmark of CML, but also from amplifications and chromosome duplications in chromosomally unstable CML cells. A second example can be found in the amplification of the thymidylate synthase gene in aneuploid cancers resistant to 5-fluorouracil[49]. Both results are intriguing because they show that a change in the micro-environment can result in an amazingly rapid replacement of all tumour cells in the population with the progeny of a variant cell that was innately resistant to the drug. This provides a dramatic illustration of the clonal evolution of tumours and a cogent lesson about how powerful CIN can be in this process. CIN probably contributes to tumour evolution in similar ways in the absence of drugs, facilitating the generation of occasional cells that have the capacity to grow more efficiently in hostile environments set up by natural host defence mechanisms.

Both the prognostic relevance of chromosomal instability and its apparent capacity to allow evasion of chemotherapeutic intervention beg for the development of rationalized therapy targeting CIN. There are at least two ways such intervention could work. One method would involve targeting chromosomal instability directly. Recent elegant experiments provide proof of principle for such a strategy with implications for inhibiting proliferation of tumour cells: reducing the concentrations of the checkpoint proteins BUBR1 or MAD2 or inhibiting BUBR1 kinase activity can provoke apoptotic cell death in aneuploid human cancer cells. Thus, suppression of mitotic-checkpoint signalling seems invariably lethal as the consequence of massive chromosome loss[50]. Both the BUB and MAD gene families were originally identified in yeast because mutations in these genes conferred resistance to mitotic arrest in response to particular pharmacological agents[43]. It is possible that anti-CIN drugs can be found by doing the exact reverse. Starting with genetic causes of chromosomal instability, high-throughput drug screens for small molecule inhibitors of chromosomal instability that act in a genotype-specific manner could be found.

Another method to target CIN would be to find agents that inhibit pathways necessary to maintain chromosomal instability. In this way, anti-CIN compounds could serve as adjunct chemotherapy, preventing cancers from acquiring the mutations that allow them to develop resistance to other cytotoxic agents.

Taking these mechanistic and clinical insights together, we anticipate that the key challenges in the next ten years will be to elucidate the complicated mechanisms underlying aneuploidy and genomic instability, and to creatively apply this knowledge to cancer therapy. We are optimistic that the role of different cellular processes in aneuploidy will lead to concerted efforts to develop compounds that will target this feature common to most, if not all, cancers. The time has come to start applying our understanding of these processes in a clinically meaningful way. If we answer this call, aneuploidy will

become known not only as one of the oldest recognized properties of cancer, but as a property integral to the development of new therapies for this disease. □

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