The consequences of tetraploidy and aneuploidy

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Summary

Polyploidy, an increased number of chromosome sets, is a surprisingly common phenomenon in nature, particularly in plants and fungi. In humans, polyploidy often occurs in specific tissues as part of terminal differentiation. Changes in ploidy can also result from pathophysiological events that are caused by viral-induced cell fusion or erroneous cell division. Tetraploidization can initiate chromosomal instability (CIN), probably owing to supernumerary centrosomes and the doubled chromosome mass. CIN, in turn, might persist or soon give way to a stably propagating but aneuploid karyotype. Both CIN and stable aneuploidy are commonly observed in cancers. Recently, it has been proposed that an increased number of chromosome sets can promote cell transformation and give rise to an aneuploid tumor. Here, we review how tetraploidy can occur and describe the cellular responses to increased ploidy. Furthermore, we discuss how the specific physiological changes that are triggered by polyploidization might be used as novel targets for cancer therapy.

Key words: Aneuploidy, Chromosomal instability, Tetraploidy

Introduction

Most malignant tumors have been found to have an abnormal karyotype with multiple structural and numerical aberrations of chromosomes – so-called 'aneuploidy'. Moreover, cancerous cells frequently contain multiple centrosomes [microtubule-organizing centers (MTOCs) (Box 1) that are required for proper chromosome segregation], which can lead to aberrant mitosis and errors in chromosomal segregation. Unusual mitoses in cells from carcinomas were observed as far back as the 19th century (von Hansemann, 1890) and for many years remained one of the most notable features of cell transformation. The observation of this phenomenon prompted Theodor Boveri to propose that missegregation of chromosomes caused by abnormal mitosis leads to aneuploidy and might be a cause of tumor development (Boveri, 2008). Systematic karyotyping of tumors revealed that the chromosome number in cancer cells is highly variable, ranging from striking hypodiploidy (considerably fewer than 46 chromosomes) to tetraploidy and hypertetraploidy (up to 200 chromosomes) (Fig. 1). Many tumors show elevated levels of chromosomal loss and gain, so-called 'chromosomal instability' (CIN), resulting in ongoing karyotypic changes (Lengauer et al., 1997) (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

The discovery of oncogenes and tumor-suppressor genes gave rise to the idea that an accumulation of specific mutations in these genes might be responsible for tumor development and that aneuploidy is a byproduct, rather than the cause, of transformation. But instead of highlighting a small number of key mutations that were responsible for tumorigenesis, new large-scale sequencing projects revealed that each particular tumor contains approximately 14 to 20 different mutant genes (http://atlasgeneticsoncology.org) (Sjoblom et al., 2006). Thus, the genomes and karyotypes of cancer cells are equally heterogeneous. At the same time, the old concept that chromosome missegregation and the associated aneuploidy might be an important step in early cell transformation has gained new experimental support (Sotillo et al., 2007; Weaver et al., 2007).

It is easy to imagine how chromosome missegregation or aberrant centrosome and spindle-pole numbers during mitosis can result in aneuploidy (Chi and Jeang, 2007; Kops et al., 2005), but an alternative and more radical mechanism might exist in which inherently instable tetraploid cells can evolve into tumorigenic aneuploid cells (Shackney et al., 1989; Storchova and Pellman, 2004). Tumorigenesis via a tetraploid intermediate might explain several observations: cancer cells frequently contain multiple centrosomes; tetraploid cells are commonly found in tumors, particularly in the early stages; and the number of chromosomes in tumor cells is often very high, which is difficult to explain by a repeated accumulation of chromosomes at each division. In this Commentary, we summarize the experimental evidence supporting the idea that tetraploid cells represent an important intermediate on the route to aneuploidy and cancer. We discuss the physiological consequences of tetraploidization and the effect of increased ploidy on CIN.

Aberrant polyploidization

Although most eukaryotic organisms are diploid (that is, they contain two sets of homologous chromosomes), cells that have more than two chromosome sets (polyploid cells) are not exceptional (Box 2). Some mammalian tissues and organs have significant numbers of tetraploid cells that arise as part of the developmental program and usually result in terminally differentiated cells (see Box 2). However, unscheduled polyploidy is not well tolerated in animals; indeed, among spontaneous miscarriages in humans with chromosomal abnormalities, triploidy and tetraploidy are responsible for approximately 20%, and this corresponds to 10% of total miscarriages (Carr et al., 1978; Eiben et al., 1990; Hassold et al., 1980; Neuber et al., 1993; Warburton et al., 1994). Furthermore, an increasing body of evidence suggests that aberrant polyploidy can trigger cell transformation (Duelli et al., 2007; Fujiwara et al., 2005).

Unscheduled tetraploidy can arise by one of three main mechanisms: cell fusion, mitotic slippage or a failure to undergo cytokinesis (Storchova and Pellman, 2004) (Fig. 2). Virus-induced cell-cell fusion was observed several decades ago in cultures by using Sendai virus (Migeon et al., 1974), and recent results

Box 1. Microtubule-organizing centers

Microtubule-organizing centers (MTOCs) nucleate and organize arrays of microtubules so that their plus ends emanate outwards. The MTOCs in yeast are called spindle-pole bodies and form a multi-layered disk that is embedded in the nuclear envelope. In higher eukaryotes, MTOCs called centrosomes are formed by two centrioles that are surrounded by the pericentriolar material (PCM). Precisely two MTOCs form a bipolar spindle, which is a crucial prerequisite for proper chromosome segregation, and each daughter cell inherits only one MTOC. The duplication of centrosomes and spindle-pole bodies occurs only once in each cell cycle, during S phase, and is tightly controlled. Supernumerary centrosomes tend to form multipolar mitosis and lead to a random distribution of chromosomes (Gisselsson et al., 2008). Accordingly, numerical centrosomal aberrations correlate with CIN in colorectal cancers (Ghadimi et al., 2000). So far, there are four known mechanisms that can generate supernumerary MTOCs (see below). Cells can employ mechanisms that enable them to cluster the extranumerary centrosomes into two poles, thus facilitating a seemingly normal bipolar mitosis. The molecular bases of these mechanisms are not well understood (see main text).

confirmed that eukaryotic cells can fuse either spontaneously in culture, after treatment with polyethylenglycol (PEG) or upon infection with a primate Mason-Pfizer monkey virus (MPMV) (Duelli et al., 2005; Duelli et al., 2007).

Tetraploid cells can also be created after an aberrant cell division. During mitosis, the chromosomes attach via proteinaceous structures called kinetochores to spindle microtubules that emanate from MTOCs (Box 1). This enables cells to segregate their chromosomes evenly into two daughter cells. Spindle-assembly checkpoint (SAC) activity holds back the onset of anaphase until all kinetochores are properly attached (Musacchio and Salmon, 2007). If there is a persistent error, the cell can escape SAC arrest (Brito and Rieder, 2006) and exit from mitosis without undergoing anaphase or cytokinesis, thereby producing a tetraploid cell with a single nucleus and two centrosomes (Azeddine et al., 1998; Lanni and Jacks, 1998). This so-called 'mitotic slippage' also occurs in cells that have an altered SAC, such as mouse embryonic fibroblasts (MEFs), which overexpress the SAC gene *Mad2* (mitotic-arrest deficient 2) (Sotillo et al., 2007).

In addition, cells that have entered anaphase might fail to finalize cell division. Cytokinesis might fail owing to a disturbance of cleavage-furrow formation, which occurs when bulk chromatin (Mullins and Biesele, 1977), or even a single lagging chromosome, is trapped in the cleavage furrow (Shi and King, 2005). The result is a single binucleated cell with two centrosomes. Abnormal spindle positioning and movements might also interfere with cytokinesis and lead to the accumulation of tetraploid cells, as has been observed, for example, in cells with deregulated integrin functions that inhibited spindle assembly (Reverte et al., 2006).

The list of mechanisms that lead to tetraploidy is growing, and raises the issue of how frequently unscheduled tetraploidization occurs in normal tissues. Although difficult to estimate, tetraploid cells can be found with variable frequencies (0.5-20%) in nearly every human tissue (Biesterfeld et al., 1994), suggesting that tetraploidization is a more common process than was previously thought. In fact, spontaneous unscheduled tetraploidization can be far more frequent than a gene mutation or chromosome-missegregation error.

Can tetraploidy trigger tumor formation?

Several lines of evidence, discussed below, suggest that unscheduled tetraploid cells that can propagate can trigger cell transformation and tumor formation. The most direct experimental evidence to date suggests that tetraploid *p53*-null mammary-epithelial-gland cells that are created by an inhibition of cytokinesis can initiate tumor formation in the nude mouse (Fujiwara et al., 2005). The cells in the tumors were near-tetraploid with significant whole-chromosomal aneuploidy and several chromosomal rearrangements. Isogenic diploid cells that underwent the identical procedure to the tetraploids and were injected into the same animals did not produce any tumors (Fujiwara et al., 2005).

Tetraploidy and oncogenes

In accordance with the tetraploid-intermediate model, defects in some genes can lead to tetraploidization, which subsequently leads to

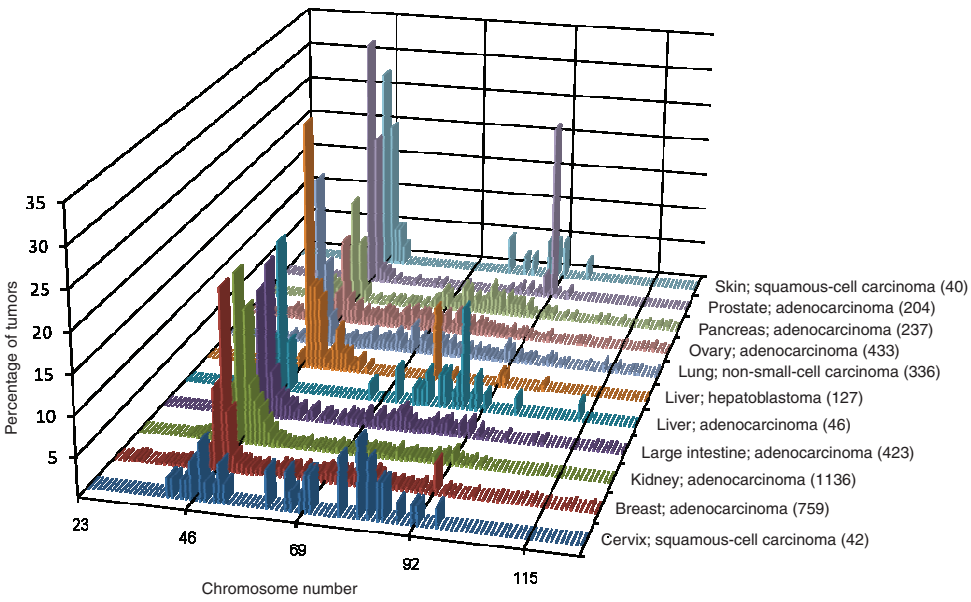

Fig. 1. Distribution of chromosome number in common cancers. The percentage of tumors plotted against the corresponding maximum chromosome number reveals that diploid or near-diploid karyotypes dominate across cancer types. A high percentage of tumors with near-triploid or near-tetraploid chromosome numbers suggests that changes in whole chromosome sets are frequent in cancers. The Mitelman Database of Chromosome Aberrations in Cancers was used as a source of the data (http://cgap.nci.nih.gov/Chromosomes/Mitelman). The bracketed numbers indicate the number of tumors analyzed for each cancer.

significant aneuploidy and tumorigenesis. In fact, mutations in some well-known oncogenes have recently been shown to induce tetraploidization. Mice that overexpress Eg5 (also known as Kif11), a member of the BimC class of kinesin-related proteins, not only accumulate tetraploid cells, but also show elevated levels of various tumors with widespread aneuploidy and genetic instability (Castillo et al., 2007). Similarly, mice that overexpress Mad2 accumulate tetraploid cells that have broken chromosomes and anaphase bridges; these mice also frequently develop tumors at between 4 and 18 months of age (Sotillo et al., 2007). Moreover, even transient Mad2 overexpression and the resulting transient CIN can initiate tumorigenesis (Sotillo et al., 2007). This observation might connect tetraploidy to a well-characterized oncogene, as Mad2 expression is upregulated in cells that have a defective retinoblastoma (Rb) pathway (Hernando et al., 2004). The overexpression of Aurora A, which results in failure of cytokinesis (Meraldi et al., 2002), can also induce CIN and the subsequent formation of mammary tumors (Wang et al., 2006). The mitotic Aurora kinases are frequently overexpressed in cancers, further substantiating the role of polyploidization and mitotic errors in carcinogenesis (Meraldi et al., 2004).

Other mutations in established oncogenes were recently linked to tetraploidization and its tumorigenic potential. For example, a defect in the gene that encodes APC (adenomatous polyposis coli; this gene is frequently mutated in aneuploid colon cancers and other tumors) results in failure of cytokinesis and subsequent tetraploidization (Caldwell et al., 2007; Dikovskaya et al., 2007). *APC* is a tumor-suppressor gene, mutations of which were identified in the early stages of gastric tumors (Fearon and Vogelstein, 1990). Its carcinogenic potential is usually associated with the Wnt and β-catenin signaling pathways (Clevers, 2006), but the effect of *APC* mutations on spindle positioning and cytokinesis appears to be independent of its interaction with β-catenin, thus suggesting a new role for APC in tumorigenesis.

Interestingly, the spontaneous tetraploidization of primary cells from patients diagnosed with Gardner syndrome was observed several decades ago (Danes, 1976). Gardner syndrome is now called familial adenomatous polyposis and is caused by hereditary mutations in *APC*. Patients develop thousands of polyps in their intestines, quickly followed by the development of colorectal cancer. It should be noted that the involvement of the Wnt pathway in the CIN seen in *APC* mutations cannot be completely excluded yet (Rusan and Peifer, 2008). The identification of separation-of-function alleles of *APC* that could distinguish between its Wnt-related and Wnt-independent functions (e.g. in spindle positioning) would clarify the role of APC in CIN.

Similarly, human cancer cells, and mouse fibroblast cells, that are deficient in the tumor-suppressor gene *BRCA2* fail to cleave at the end of mitosis, and accumulate binucleate tetraploids and polyploids both in vivo and in vitro (Daniels et al., 2004). The tetraploids that are created by cell-cell fusion owing to virus infection can also propagate and become oncogenically transformed if even just one of the fusion partners expresses an oncogene or mutated *p53* tumor-suppressor gene (Duelli et al., 2005).

Tetraploidy in vivo

Polyploid cells are frequently found in tumors of all stages (Fig. 1), and several in vivo observations support the idea that tetraploid cells occur as an early step in tumor formation. Cell fusion induced by SV40 (simian virus 40) in pancreatic cells leads to the accumulation of tetraploid cells with the subsequent appearance of aneuploid cells and neoplastic tissues (Ornitz et al., 1987). In a pre-malignant condition called Barrett's esophagus, tetraploid cells can be detected before gross aneuploidy occurs (Barrett et al., 2003; Galipeau et al., 1996; Maley, 2007). Tetraploidy and CIN occur during the early stages of cervical carcinogenesis, predisposing cervical cells to the formation of aneuploidy (Olaharski et al., 2006).

So how can tetraploidy promote tumorigenesis? First, tetraploidy appears to render an increase in CIN in eukaryotic cells (Cowell and Wigley, 1980; Mayer and Aguilera, 1990; Storchova et al., 2006). Second, a diploid cell with increased CIN would probably die after losing multiple chromosomes in an aberrant mitosis, whereas a polyploid cell might have a higher chance of survival owing to a greater redundancy in chromosomal content. This might serve to buffer the damaging effects of chromosome loss following multipolar mitosis and produce progeny that, although grossly aneuploid, remain viable (Shackney et al., 1989; Storchova and Pellman, 2004). The unbalanced gene expression of the aneuploid cells can further accelerate CIN. Interestingly, in most of the examples mentioned above, tumor development is triggered by the combination of tetraploidy and an additional mutation – p53 deficiency or the overexpression of Mad2 or Eg5. The additional

Box 2. The advantages and disadvantages of polyploidy

In general, two different types of polyploidy can be recognized. Allopolyploids arise by the fusion of two or more cells of distantly related genomes – for example, of two different species. Autopolyploids, however, arise by the duplication of a single genome or by fusion of closely related genomes, albeit not necessarily from the same individual. Autopolyploidy, which is the focus of this article, occurs in all eukaryotes, but its frequency and consequences are less well known. In several tissues, the formation of polyploid cells is a part of the developmental program. These polyploids typically arise by the process of endomitosis or cell fusion and usually represent the terminally differentiated stage, e.g. megakaryocytes or embryonic trophoblasts (Edgar and Orr-Weaver, 2001). The relatively frequent presence of autopolyploid cells in normal tissues (0.5-20% depending on the tissue) suggests that polyploidy brings some advantages to the organism (Biesterfeld et al., 1994).

It is often suggested that polyploidy affects cellular metabolic rates and might be a physiological response to metabolic stress (Storchova and Pellman, 2004). For example, hepatocytes of newborn mammals are predominantly diploid, but become more and more tetra- and octa-ploid during the ageing process (Guidotti et al., 2003). Liver-challenging circumstances, such as partial hepatectomy or drug intoxication, also increase the polyploidization rate, further supporting this hypothesis (Fausto and Campbell, 2003). Moreover, owing to its effect on cellular size (cells with increased ploidy are bigger), polyploidization might be a simple way to regulate tissue and organ size (Otto, 2007).

By contrast, polyploidy has its costs, as is documented by the fact that whole-organism polyploidy is not tolerated in most mammals. Moreover, increased ploidy is associated with alterations in chromosome stability, leading to abnormal chromosomal numbers – so-called aneuploidy (Otto, 2007; Shackney et al., 1989; Storchova and Pellman, 2004). It should be also noted that polyploidization played an important role during the evolution of eukaryotes (Otto, 2007). Although considerable progress has been made recently, the effects of ploidy on the physiology of eukaryotic cells remain enigmatic (Comai, 2005).

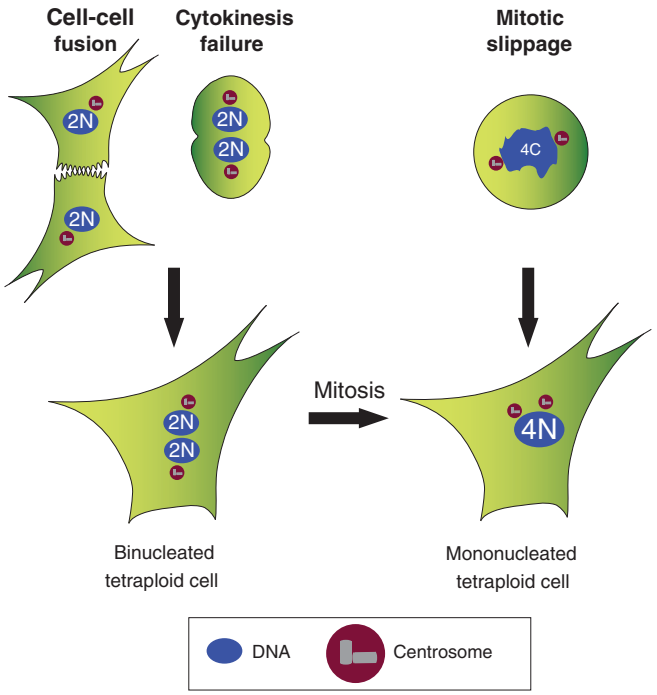

Fig. 2. The three main roads to tetraploidy. Cell-cell fusion and failure of cytokinesis generate binucleated cells that contain two centrosomes. Binucleated cells can form mononucleated tetraploids after successful passage through the next mitosis. Mitotic slippage is a cellular adaptation to persistent mitotic arrest. Cells bypass anaphase, telophase and cytokinesis, and progress into the next G1 phase without correcting the mitotic error that triggered the arrest. Cells that are derived from mitotic slippage contain a single tetraploid nucleus that is accompanied by two centrosomes. 2N, diploid nucleus; 4N, tetraploid nucleus; 4C, diploid nucleus with replicated chromosomes.

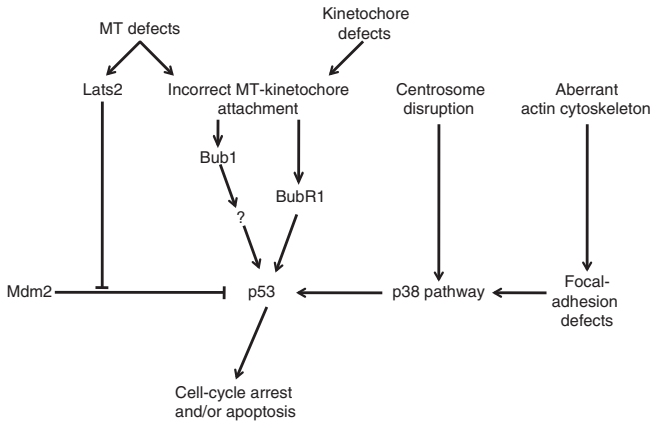

Fig. 3. Defects associated with aberrant cell division can trigger cell death and might prevent proliferation of tetraploid cells. Defective kinetochores and microtubules (MTs), as well as disruption of centrosomes or the actin cytoskeleton, can initiate cell death. The mitotic-checkpoint proteins Bub1 and BubR1 might also trigger a post-mitotic, p53-dependent cell death after chromosome missegregation owing to spindle defects. The centrosomal kinase Lats2 inhibits p53 degradation by inhibiting Mdm2 in the absence of MTs, thus activating the apoptotic pathway. Disruption of centrosome integrity induces the p38 stress pathway, which can also trigger p53-dependent apoptosis. The experimental formation of tetraploid cells is frequently associated with disruption of the actin cytoskeleton. Cytoskeletal defects lead to disrupted focal adhesions, which, in their unimpaired state, are essential for cellular survival pathways because they can suppress the p38 stress pathway. p53 mediates apoptotic and cell-cycle-arresting signals by initiating the transcription of multiple effector proteins. It should be noted that the proposed pathways in this figure are not well established. For further details, see text.

mutations might be important because they can trigger tetraploidization or allow the propagation of arising tetraploids. Alternatively, they might have a more direct role in tumorigenesis. Experiments that could distinguish between the involvement of genetic mutations, polyploidy and aneuploidy in tumorigenesis will be a crucial challenge in future investigations.

Restricting the growth of polyploid cells

Considering that tetraploid cells are highly unstable and prone to transformation, one could expect that there exists an active mechanism to avoid proliferation of aberrantly arising tetraploid cells (Ganem and Pellman, 2007). Indeed, proliferation of tetraploid cells that are created by interfering with the actin cytoskeleton, which plays an essential role in cytokinesis, is usually held in check. The arrest usually occurs in the G1 phase following an aberrant mitosis and requires p53, p21, p16 or Rb function. Most of the resulting tetraploid cells undergo apoptosis (Andreassen et al., 2001; Cross et al., 1995; Fujiwara et al., 2005; Meraldi et al., 2002). Accordingly, cells that lack p53 or a functional apoptotic pathway accumulate tetraploids that spontaneously arise in culture (e.g. Cross et al., 1995). Thus, it has been proposed that there is an active checkpoint that prevents the proliferation of tetraploid cells in normal tissues, a so-called 'tetraploidy checkpoint' (Margolis et al., 2003). Recent experiments have demonstrated that the G1 arrest of tetraploid cells was most probably a consequence of the high concentrations of drugs used to abort cytokinesis. Indeed, when low concentrations of actin-depolymerizing drugs were used, tetraploid cells were generated from several different cell lines and progressed into the next cell cycle (Uetake and Sluder, 2004; Wong and Stearns, 2005). Furthermore, some naturally occurring polyploid cells can proliferate (e.g. in the liver) (Fausto and Campbell, 2003; Guidotti et al., 2003).

Signals that trigger the arrest of tetraploids

Current evidence suggests that the increased numbers of chromosomes, centrosomes or even nuclei do not trigger the p53-dependent arrest and subsequent death of tetraploids in the next cell cycle. However, the survival rate of tetraploid cells is usually low and most of them do indeed arrest in G1 (Andreassen et al., 2001; Cross et al., 1995; Fujiwara et al., 2005; Livingstone et al., 1992). What is the signal that triggers the arrest in tetraploid cells regardless of their origin? One possibility is that abnormal mitosis seriously damages the mitotic apparatus and/or cytoskeleton, which in turn activates the checkpoint response. Second, aberrant mitosis can cause DNA damage that then triggers G1 arrest and, eventually, death.

Several lines of evidence suggest that damage to the spindle or microtubule and actin cytoskeleton can cause a cell-cycle arrest (Fig. 3). Direct damage to a centrosome or induction of centrosomal stress leads to the activation of the p38 stress-response pathway, a p53-dependent G1 arrest and subsequent apoptosis (Mikule et al., 2007). One potential player in this process is the centrosomal kinase Lats2 (large tumor suppressor homolog 2), as Lats2-deficient cells that escape from mitotic arrest can proliferate as tetraploids. The existing evidence suggests that Lats2 physically interacts with the ubiquitin ligase MDM2 and inhibits its ability

to negatively regulate p53. Thus, the absence of Lats2 results in functional p53 deficiency (Aylon et al., 2006).

Nonspecific cellular stress owing to a dysfunction of the mitotic apparatus might also induce a G1 arrest. The SAC protein Bub1 appears to be a good candidate to mediate p53 activation, as reduced Bub1 function induces cellular senescence in p53-proficient cells, whereas it does not in cells that express a dominant-negative p53 mutant (Gjoerup et al., 2007). Bub1 also mediates death after aberrant mitosis in MEFs. Whereas wild-type MEFs die within a few hours following mitosis with chromosome missegregation, their survival is significantly increased in cells with reduced Bub1 levels, and the frequency of apoptosis correlates with the expression levels of Bub1 (Jeganathan et al., 2007). Although the association of Bub1 and p53 in triggering post-mitotic arrest is intriguing, we have much to learn about the underlying processes. Another SAC protein, BubR1 (also known as Bub1b), which has been found to be downregulated in some adenocarcinomas, can be involved in triggering post-mitotic cell death after aberrant mitosis, as the spindle disruption in cells that lack BubR1 generates proliferating tetraploids (Shin et al., 2003). It has recently been proposed that BubR1 is important for the phosphorylation and stabilization of p53 (Ha et al., 2007).

Other experimental evidence suggests that the length of mitosis rather than microtubule damage determines post-mitotic arrest in mammalian tetraploids (Uetake and Sluder, 2007). As no transcription occurs in mammalian cells during mitosis (Gottesfeld and Forbes, 1997), it is possible that the prolonged period spent in mitosis without any transcription can trigger subsequent cell death (Blagosklonny, 2006). DNA damage, particularly chromosome breaks that are acquired during aberrant mitosis or a prolonged block in mitosis, can also trigger a G1 arrest and apoptosis in newly arising tetraploid cells. Both Chinese-hamster and human-embryonic fibroblasts create DNA damage after a nocodazole-induced mitotic arrest (Dalton et al., 2007; Quignon et al., 2007). The DNA damage appears in all cells that undergo prolonged mitotic arrest, regardless of whether they escape the arrest (and become tetraploid) or successfully divide. Thus, it is rather improbable that the damage would activate apoptosis only in tetraploid cells.

We still do not understand what triggers the death of newly arising tetraploids. It is likely that there is not one specific pathway that is responsive to tetraploidy, but rather that several cellular defects that are caused by aberrant mitosis and polyploidization can trigger cell death depending on the cellular context (Fig. 3). An interesting insight might be provided by the analysis of developmentally programmed tetraploids, as similar mechanisms might apply in both aberrant and scheduled tetraploidization. For example, it was recently shown that a deficiency in p53 increases the level of polyploidization in megakaryocytes, which become highly polyploid in the developmentally programmed process of thrombocyte formation (Fuhrken et al., 2008).

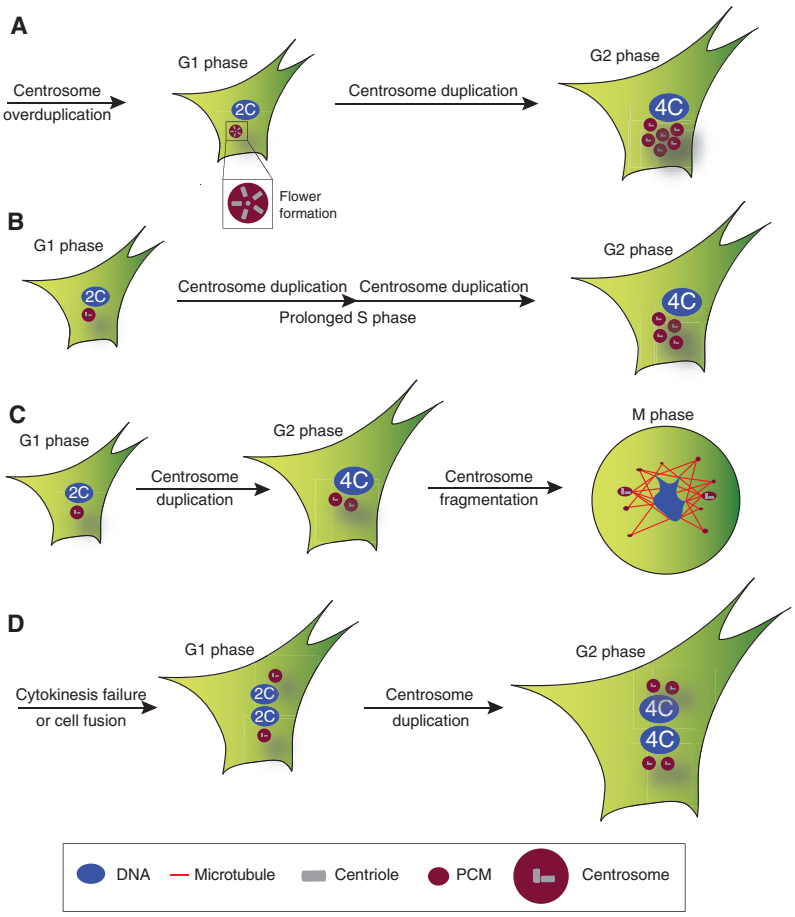

Fig. 4. Centrosome amplification. Centrosome amplification can occur by at least four different mechanisms. (A) If the copy-number control fails (overduplication), e.g. owing to overexpression of polo-like kinase 4 (Plk4), several daughter centrioles are formed around one mother (flower formation). This leads to multiple centrosomes in the next cell cycle (Kleylein-Sohn et al., 2007). (B) Certain cancer cell lines, such as CHO or U2OS, duplicate their centrosomes more than once per cell cycle if kept in a prolonged S phase (reduplication) (Kuriyama et al., 2007). A similar effect can be observed in *Xenopus laevis* egg extracts arrested with an inhibitor of DNA synthesis (Hinchcliffe et al., 1999). (C) Pericentriolar material (PCM), the fibrous network surrounding centrioles, can fragment if the centrosomal structure is impaired by the inhibition, depletion or overexpression of centrosomal proteins. The acentriolar fragments can still serve as MTOCs and create multipolar spindles (Oshimori et al., 2006). (D) Tetraploid cells contain two centrosomes in G1 phase regardless of the mechanism of their formation. The centrosomes are duplicated in the subsequent S phase (e.g. Meraldi et al., 2002). 2C, diploid nucleus with unreplicated chromosomes; 4C, diploid nucleus with replicated chromosomes.

Chromosomal instability in tetraploid cells

Tetraploidy instigates high CIN in yeast and mammalian cells (Fujiwara et al., 2005; Mayer and Aguilera, 1990; Storchova et al., 2006), but what is the underlying mechanism? At least for mammalian cells, the supernumerary centrosomes were proposed as the major source of instability (Boveri, 2008; Nigg, 2002), as the newly formed tetraploid cells contain – in addition to twice the number of chromosome sets – two extra centrosomes (Fig. 2).

Supernumerary centrosomes can arise by several different means, either in diploid cells or through the formation of a tetraploid cell (Fig. 4). The presence of multiple centrosomes can then lead to the formation of multipolar spindles and, consequently, a defect in chromosome segregation. This significantly impairs progression through mitosis (owing to the activation of the SAC)

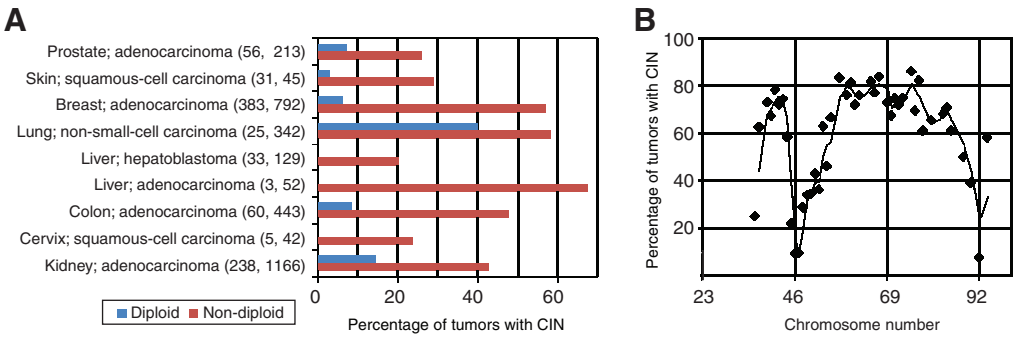

Fig. 5. Numerical CIN in various cancers. (A) Non-diploid tumors display CIN much more often than diploid tumors. The percentage of diploid (blue) and non-diploid (red) tumors with cell-to-cell variability in chromosome number has been plotted. Bracketed numbers indicate the number of tumors analyzed for diploid and non-diploid tumors, respectively. (B) Numerical CIN is less frequent in diploid and tetraploid tumors than in aneuploid tumors. The percentage of tumors with CIN is plotted against the average chromosome number. Every data point represents at least five tumors. The trend line represents the moving average in the second period (i.e. each point of the trend line represents the average of the two neighboring data points). The Mitelman Database of Chromosome Aberrations in Cancers was used as a source of the analyzed data (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

and, as a result, the multipolar mitoses take longer (Basto et al., 2008; Gisselsson et al., 2008; Kwon et al., 2008; Yang et al., 2008). Multipolar mitoses have been shown to result in high CIN owing to unsynchronized sister-chromatid separation, a high frequency of non-disjunction and the occurrence of diplochromosomes (Gisselsson et al., 2008).

Nonetheless, tetraploid budding-yeast cells show increased CIN even without multipolar spindles, as the spindle-pole bodies fuse upon tetraploid formation. Here, the increased CIN is mainly a consequence of frequent improper microtubule-kinetochore attachments (mostly syntelic attachments, in which both sister kinetochores are attached to the same spindle pole). The elevated occurrence of syntelic attachments is probably attributable to the altered spindle geometry in yeast tetraploids. The cellular and nuclear volume, as well as the surface area of the spindle-pole body, doubles with doubling ploidy, whereas the spindle length remains similar from haploids through to tetraploids (Storchova et al., 2006). Currently, it is unclear whether similar altered spindle geometry exists in human cells as well.

How can increased ploidy promote the chromosomal rearrangements, translocations or amplifications that are so often observed in cancer cells (http://cgap.nci.nih.gov/Chromosomes/Mitelman)? As the chromosomal rearrangements are generally thought to be a result of improper DNA-damage repair, we can envisage two major sources of rearrangements. First, tetraploids accumulate an increased amount of spontaneous DNA damage. The simple fact that there is double the amount of DNA means that there will be twice the amount of spontaneous DNA damage and hence an increased requirement for, or even a saturation of, DNA-repair processes, as was shown for budding-yeast tetraploids (Mable and Otto, 2001; Storchova et al., 2006). Moreover, abnormal mitosis and prolonged mitotic arrest in human cells leads to the accumulation of DNA breaks (Quignon et al., 2007). Chaotic multipolar mitosis can also break chromosomes directly. This might be unlikely in cells with intact chromosomes, as the spindle forces are not strong enough to break the DNA backbone (Nicklas et al., 2001); it might become more feasible, however, if there are nicks or single-stranded gaps present in chromosomes, and each chromatid is attached by more than ten microtubules (Jannink et al., 1996). Moreover, DNA breakage might occur during aberrant cytokinesis under the mechanical action of the cleavage furrow (Jannink et al., 1996).

Second, even if the amount of DNA damage does not increase significantly, the processes that are required for repair might be less efficient in tetraploids. Indeed, we have shown an accumulation of DNA damage in wild-type yeast tetraploid cells (Storchova et al., 2006), and have found that its repair takes longer (Z.S., unpublished results). Moreover, both yeast and mammalian tetraploid cells appear to be more sensitive to agents that damage DNA than are isogenic diploids (Hau et al., 2006; Storchova et al., 2006). Although these mechanisms can lead to instability, it should be noted that tetraploid cells still represent a more stable state than any other aneuploidy (Fig. 5). Thus, it is possible that tetraploidy provides a small increase in instability that is still compatible with survival but is sufficient enough to generate new genomic variants.

Arguably, having abnormal numbers of chromosomes, possibly achieved through a genome-duplication event, might often be a burden to eukaryotic cells and instigate several physiological changes. The ability of cells to adapt to these changes, however, can give rise to cells with higher fitness than the parental cells. The role of CIN, polyploidy and aneuploidy in tumorigenesis is clearly highly complex (Fig. 5). This is reflected by the fact that an experimentally induced aneuploidy in a CENP-E knock-out mouse can result in both tumorigenesis and tumor suppression (Weaver et al., 2007). Similarly, mice with a graded decrease of Bub1 start to develop spontaneous tumors after Bub1 levels are reduced beyond a certain threshold, whereas Bub1-haploinsufficient mice are not prone to tumors (Jeganathan et al., 2007).

Adaptation to polyploidy and aneuploidy – new insights for cancer therapy

CIN is common in many tumors, yet a significant number of tumors propagate with an aberrant but stable karyotype (Fig. 5). This implies that the period of instability in these cells was only transient and that the unstable cells evolved a single clone with significant proliferative advantages that eventually outgrew the original population. The mechanism by which a highly unstable clone evolves into a stably propagating aneuploid karyotype is currently not understood, but probably involves adaptations that can control CIN.

One important step towards adaptation is observed in cells with multiple centrosomes; these cells can suppress spindle multipolarity, either by functional silencing of extra centrosomes or by clustering of the multiple centrosomes into two functional spindle poles (Basto

et al., 2008; Brinkley and Goepfert, 1998; Quintyne et al., 2005). Several factors were found to be important to prevent multipolarity. For example, overexpression of the spindle protein NUMA1 (a protein important for spindle formation and stabilization) affects localization of a motor-protein complex and subsequently causes multipolarity (Quintyne et al., 2005). A recent genome-wide RNAi screen, designed to identify mechanisms that are required for efficient centrosome clustering in *Drosophila melanogaster* cells with supernumerary centrosomes, confirmed the involvement of a number of genes that promote the bundling of spindle microtubules. The screen also unexpectedly identified several genes that are involved in the SAC, actin regulation, cell polarity and cell adhesion (Kwon et al., 2008). Apparently, suppressing multipolar spindles is a complex process that requires the coordination of the actin cytoskeleton with intrinsic spindle forces. Importantly, it has been convincingly demonstrated that blocking centrosome clustering and promoting multipolar mitosis can selectively kill cells with multiple centrosomes, as the knockdown of a gene encoding a minus-end-directed kinesin called HSET (also known as KIFC1) – the human homolog of one of the identified genes – did not affect the viability of diploid cells with two centrosomes, but killed more than 90% of cells with multiple centrosomes (Kwon et al., 2008).

A recent genome-wide screen in budding yeast revealed a group of 39 genes that are specifically required for the survival of cells with increased ploidy (Storchova et al., 2006). Most of these so-called 'ploidy-specific lethal' genes are involved in mitotic-spindle function, homologous recombination and sister-chromatid cohesion, pathways that have all been implicated in the maintenance of genomic stability. These findings demonstrate that increased ploidy alters the physiology of eukaryotic cells so significantly that it even alters their genetic requirements. Other phenotypic characteristics – for example, chromosome-loss rates or sensitivities to various toxic agents – are altered in isogenic strains that differ only in ploidy. Although more experimental evidence will be needed, one plausible explanation is that the altered geometry of tetraploid cells affects their physiology. The results remind us that not only gene mutations can affect cellular phenotype, but the actual physical characteristics of each cell can influence the behavior. Understanding the role of intracellular geometry, as well as the effect of cell size and shape, on physiological processes should become an important future direction of cell biology.

Uncovering the physiological consequences of polyploidy and aneuploidy, as well as the types of cellular adaptations that are necessary for the survival of cells with an abnormal number of chromosomes, might provide new insight into the molecular mechanisms that underlie tumorigenesis. Moreover, targeting the genes that are involved in centrosomal clustering or ploidy-specific lethal genes could represent new and interesting possibilities for cancer therapy.

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