The Causes and Consequences of Polyploidy in Normal Development and Cancer

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Abstract

Although nearly all mammalian species are diploid, whole-genome duplications occur in select mammalian tissues as part of normal development. Such programmed polyploidization involves changes in the regulatory pathways that normally maintain the diploid state of the mammalian genome. Unscheduled whole-genome duplications, which lead primarily to tetraploid cells, also take place in a substantial fraction of human tumors and have been proposed to constitute an important step in the development of cancer aneuploidy. The origins of these polyploidization events and their consequences for tumor progression are explored in this review.

Contents

MAINTENANCE OF THE DIPLOID MAMMALIAN GENOME

Mammals are diploid. The only exception to this rule is the red vizcacha rat (*Tympanoctomys barrerae*) and its close relatives, which are tetraploid (Gallardo et al. 1999). In other mammals, tetraploidy causes early lethality and spontaneous abortion or resorption of the embryo (Creasy et al. 1976, Kaufman 1991). In contrast, tetraploid species are widespread in plants and fairly frequent in insects, crustaceans, fish, amphibians, and reptiles (Masterson 1994, Otto & Whitton 2000). Polyploidization has been proposed to have occurred twice early in vertebrate evolution (Ohno 1970, Van de Peer et al. 2009). The resulting paleopolyploid state of the mammalian genome can explain the multiple paralogs of many genes (e.g., Hox gene cluster; Ras, Rb, and E2F genes) (Schughart et al. 1989). Nonetheless, each mammalian somatic cell generally contains exactly two copies of the autosomal chromosomes (the maternal and paternal homologs).

The diploid state of mammalian somatic cells is guarded by control mechanisms that act throughout the cell cycle to prevent the occurrence and/or proliferation of cells with deviant DNA contents (**Figure 1**). The cyclin-dependent kinases (Cdks) and their cyclin subunits regulate key protein phosphorylation events such that DNA replication and mitosis alternate, driving the cell cycle forward; the initiation of DNA replication is controlled by a system that prevents rereplication; and two types of checkpoints [the DNA damage checkpoints and the spindle assembly checkpoint (SAC)] ensure the integrity of the diploid genome and the correct separation of newly replicated sister chromatids. These controls are highlighted in **Figure 1** and discussed briefly below.

Chromosome nondisjunction is prevented by regulatory pathways, generally known as error correction pathways, that promote correct kinetochore-microtubule attachments (for a review see Musacchio & Salmon 2007). The SAC exerts an additional level of control on the cell cycle in that it blocks anaphase until all chromosomes have achieved bioriented attachment to the mitotic spindle (**Figure 1**). The dissolution of sister chromatids in anaphase requires activation of the APC/C (anaphase-promoting complex/cyclosome), an ubiquitin ligase that together with either Cdc20 or Cdh1 targets mitotic proteins for degradation by the proteasome. A signal emanating from unattached kinetochores inhibits APC/C-Cdc20. Once all chromosomes are correctly aligned on the spindle, the SAC is switched off, and APC/C-Cdc20 becomes active, which leads to degradation of securin. With securin gone, separase can cleave the cohesin that holds the sister chromatids together, and one copy of each duplicated chromosome is segregated to each daughter cell.

After mitosis, a regulatory pathway controls the initiation of DNA replication such that

Tetraploidy: a form of polyploidy; the state of having four sets of chromosomes (4N)

E2F: transcription factor that promotes S-phase entry and is negatively regulated by Rb

Anaphase-promoting complex/cyclosome (APC/C): an E3 ubiquitin ligase bound to the activator Cdc20 in metaphase and to Cdh1 in late anaphase and G1

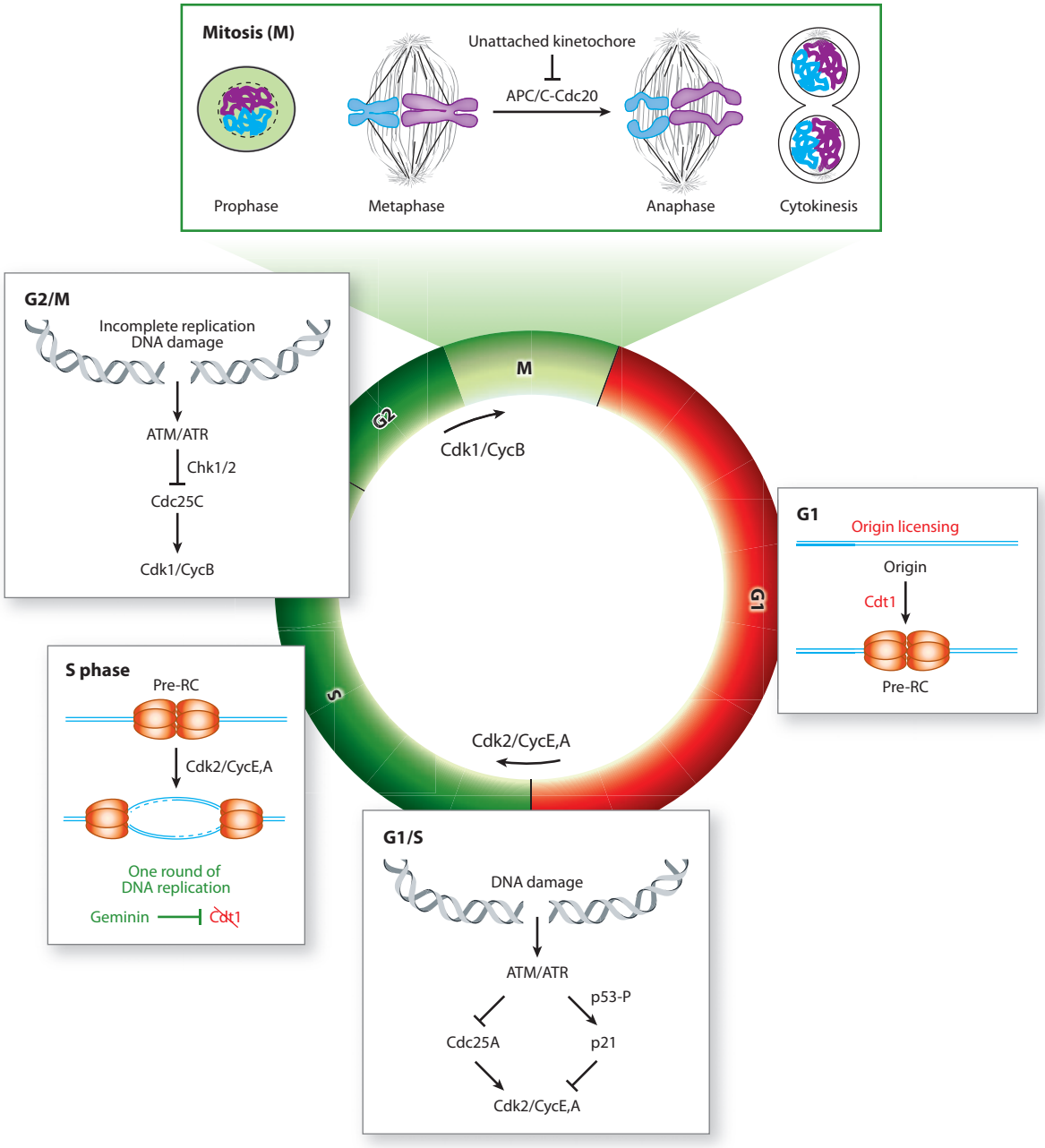

Figure 1

How mammalian somatic cells maintain a diploid genome. The schematic outlines the normal somatic cell cycle and highlights the three main mechanisms by which the diploid genome is kept intact: assurance of correct chromosome segregation in mitosis, regulation of DNA replication, and the DNA damage checkpoints. Abbreviations: APC/C, anaphase-promoting complex/cyclosome; ATM/ATR, ataxia telangiectasia mutated/ATM and Rad3-related; Cdk, cyclin-dependent kinase; Chk, checkpoint kinase; Cyc, cyclin; pre-RC, prereplication complex.

each sequence is replicated only once during the following S phase (**Figure 1**) (for a review see Remus & Diffley 2009). Replication origins are licensed at the end of mitosis and in the beginning of G1 through the formation of the prereplication complex (pre-RC). The formation of the pre-RC requires Cdt1, which accumulates in G1 and is degraded upon entry into S phase after an ubiquitinylation step that depends on the initiation of DNA replication. In S and G2, origin relicensing is prevented both by the degradation of Cdt1 and the expression of geminin, an inhibitor of Cdt1. This system is reset by the APC/C-Cdh1-mediated degradation of geminin at the end of mitosis and the rise of Cdt1 in G1. As a result, replication origin licensing is coupled to progression through mitosis.

The DNA damage response pathways monitor the integrity of the diploid chromosome complement (see for a review Ciccia & Elledge 2010). Two related phosphoinositide 3-kinase-like protein kinases, the ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related) kinases, can initiate a DNA damage checkpoint. The ATM kinase is activated by double-stranded DNA breaks (DSBs), whereas ATR responds to pathological single-stranded DNA (ssDNA), including ssDNA generated by DNA replication problems or resection of a DSB. ATM and ATR phosphorylate and activate downstream effector kinases [checkpoint kinase 2 and 1 (Chk2 and Chk1), respectively], which in turn phosphorylate proteins that enforce cell cycle arrest. Replication of damaged DNA, a potentially deleterious event, is prevented by blocking cell cycle progression into S phase. This is achieved through inactivation of the Cdc25A phosphatase (which is required for activation of Cdk2) and activation of the p53 pathway (resulting in upregulation of the Cdk inhibitor p21). Because the effect of Cdc25A inactivation is transient, permanent arrest in G1 and induction of senescence and/or apoptosis is absent from p53-deficient mouse cells. In human cells, however, DNA damage also results in upregulation of p16, which can induce a G1 arrest through preventing the phosphorylation of Rb (reviewed in Gil & Peters 2006). In G2, incompletely replicated DNA and broken chromosomes activate the G2/M checkpoint. In this setting, ATM and/or ATR signaling delays entry into mitosis primarily by inactivating Cdc25C, which is needed for the activation of Cdk1/CycB. In p53/Rb-deficient cells, this is the main checkpoint to prevent cell division in the presence of DNA damage.

In addition to kinetochores and replication start sites, a third chromosomal element, the telomere, plays a critical role in the stability of the diploid chromosome complement (for a review see de Lange 2009). Telomeres contain a protein complex, called shelterin, that prevents the activation of the ATM and ATR DNA damage checkpoints at the natural ends of chromosomes (Palm & de Lange 2008). In addition, shelterin represses inappropriate DNA repair reactions at telomeres such as end-joining pathways and homology-directed repair. The binding site for shelterin, the telomeric repeat DNA, is maintained by telomerase (see for a review Cong et al. 2002). In the absence of telomerase, telomeres gradually shorten and eventually become dysfunctional. Such dysfunctional telomeres are recognized as sites of DNA damage, which leads to the activation of the ATM and ATR signaling pathways and induction of permanent arrest through p53-mediated upregulation of p21 and, in human cells, the p16/Rb pathway. Chromosomes lacking functional telomeres owing to telomere shortening or because of experimental inactivation of shelterin undergo end-to-end fusions, which result in dicentric chromosomes that often missegregate or break during mitosis.

TETRAPLOIDIZATION IN NORMAL DEVELOPMENT

Despite these safeguards, the conversion from diploidy to polyploidy is part of normal mammalian development and differentiation in at least three specialized cell types (**Figure 2**).

A striking case of developmental polyploidization is the placental trophoblast giant

Cdt1: mediates licensing of replication origins through the formation of the prereplication complex

Geminin: replication inhibitor that binds and inhibits Cdt1; it is normally expressed in S/G2 and degraded in mitosis

Ataxia telangiectasia mutated (ATM) and ATM and Rad3-related (ATR): phosphoinositide 3-kinase-related protein kinases that mediate the DNA signal

Checkpoint kinase 1 and 2 (Chk1/Chk2): Ser/Thr effector kinases that mediate cell cycle checkpoints in response to ATM and ATR

Polyploidy: the state of having an aberrantly high DNA content composed of an integer multiple of the diploid set of chromosomes

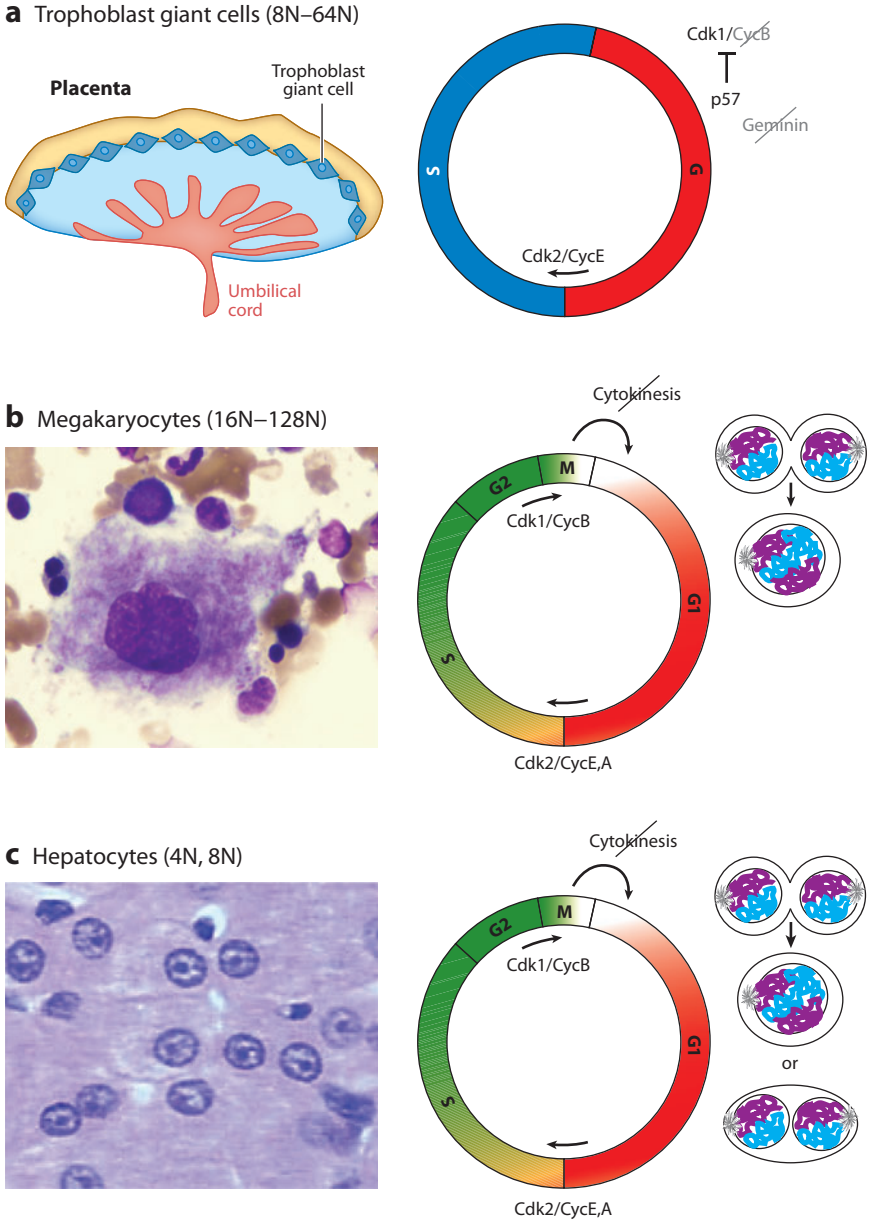

Figure 2

Examples of developmentally programmed polyploidization. Three mammalian cell types exhibit prominent polyploidization under physiological conditions: (*a*) trophoblast giant cells, (*b*) megakaryocytes, and (*c*) hepatocytes. The schematics on the right show the alterations in the cell cycle that allow polyploidization to occur. Drawing of trophoblast giant cells is adapted from Rossant & Cross (2001); image of megakaryocytes is from **http://blass.com.au/definitions/megakaryocyte**; and image of hepatocytes is from **http://www.vivo.colostate.edu/hbooks/pathphys/digestion/liver/histo_hcytes.html.** Abbreviations: Cdk, cyclin-dependent kinase; Cyc, cyclin.

cell (TGC), which can reach a DNA content of up to 8N to 64N (Ullah et al. 2009a,b). TGC polyploidization begins with a differentiation step induced when FGF4 signaling abates. This increases the expression of the Cdk inhibitor p57 (Ullah et al. 2008), which, together with a reduction in the translation of Cyclin B (Palazon et al. 1998), blocks Cdk1/CycB-mediated mitosis. The ensuing endocycles appear to involve oscillation of Cdk2/CycE activity and p57 level (Hattori et al. 2000), which induces alternating S and G phases. TGCs have reduced amounts of geminin (Gonzalez et al. 2006), which may be a prerequisite for pre-RC formation in these amitotic cycles. Because no mitosis occurs between S phases, the replicated chromatids are not separated, so they form cohesin-linked polytene chromosomes similar to those of *Drosophila* salivary glands (Ilgren 1981). The endocycles have been proposed to accelerate TGC growth by shortening the cell cycle and simultaneously increasing the number of chromosomes (Gardner & Davies 1993, Gonçalves et al. 2003).

Whereas TGCs skip mitosis altogether, megakaryocytes become polyploid through an abortive mitosis (endomitosis) in which anaphase and telophase can occur but cytokinesis fails (**Figure 2**). The abortive mitosis appears to be due to suboptimal levels of Cdk1/CycB activity and premature degradation of Cyclin B (Ravid et al. 2002). Although the clusters of duplicated chromosomes temporarily separate and a midzone forms, the cleavage furrow regresses, and all chromosomes eventually end up in a single nucleus. The resulting cell is in a G1-like state, progresses through S phase, and enters another round of endomitosis; the process is repeated several times to form polyploid cells up to 128N (modal ploidy of 16N) (Winkelmann et al. 1987).

A third example of polyploidization involving an abortive mitosis occurs in hepatocytes (reviewed in Celton-Morizur & Desdouets 2010; **Figure 2**). During liver growth, the frequency of tetraploid (4N) and octoploid (8N) hepatocytes progressively increases (Watanabe et al. 1978). These cells often have two 2N (or 4N) nuclei and arise from a failure in cytokinesis (Celton-Morizur et al. 2010, Guidotti et al. 2003, Margall-Ducos et al. 2007). Multinucleated hepatocytes can also arise from cell-cell fusion (Duncan et al. 2009), and hepatocytes with a single 4N or 8N nucleus arise readily when anaphase is inhibited or telomeres are damaged (Lazzerini Denchi et al. 2006, Wirth et al. 2006). Hepatocytes are particularly prone to increasing their ploidy in response to DNA damage, whereas most other cells undergo apoptosis. This attribute may be related to the altered manner in which the hepatocyte p53 pathway responds to DNA lesions (Fei et al. 2002).

A fourth type of polyploidization is seen in skeletal muscle and osteoclasts in which cell fusion generates polynucleated terminally differentiated cells (Vignery 2000, Yaffe & Feldman 1965). Polyploidy has also been observed sporadically under physiological conditions in lactating mammary gland, urothelium, mesothelium, and Purkinje neurons (Biesterfeld et al. 1994), but how these whole-genome duplications arise is less clear.

The benefits of programmed polyploidy are not fully understood. Polyploidy results in a decreased surface-to-volume ratio for both the whole cell and the nucleus, thereby minimizing membrane requirements but also lowering the efficiency of transport (Comai 2005, Henery et al. 1992). Polyploid cells, including hepatocytes and placental TGCs, often provide nutrients and metabolites. It has been proposed, therefore, that polyploidization increases the metabolic capacity of a tissue, possibly by funneling energy toward gene duplication and protein synthesis instead of cell division and membrane synthesis (Joubes & Chevalier 2000, Lee et al. 2009). Although megakaryocytes do not have a nourishment function, they can be viewed similarly as a stockpile of components needed to generate platelets. For example, a 32N megakaryocyte can give rise to ~3,000 platelets (Ravid et al. 2002). Assuming that for every twofold increase

in ploidy an approximately twofold increase in volume occurs (Henery et al. 1992), at least 16 diploid megakaryocytes would be necessary to deliver the same number of platelets. Considering the diminished requirement for membrane synthesis in a single 32N cell versus 16 diploid cells, polyploidization of megakaryocytes might be an efficient way to direct membrane synthesis toward the production of platelets and to avoid the energy expenditure of cell divisions.

Recently, Duncan et al. (2010) proposed that tetraploidization of hepatocytes has the benefit of generating genetic variability. During liver regeneration, tetraploid hepatocytes can undergo a reductive mitosis to create aneuploid daughter cells with a reduced chromosome number (Duncan et al. 2010). These aberrant divisions are most likely the consequence of multipolar mitoses generated by supernumerary centrosomes. As the resulting aneuploid cells could carry reassorted alleles, this genetic variation might increase the chance of resistance to exogenous stress.

TETRAPLOIDIZATION WITH AGING AND STRESS

In several tissues, tetraploid or octoploid cells appear with aging and under pathological conditions. For instance, the incidence of polyploidy increases with age in hepatocytes and vascular smooth muscle cells (Celton-Morizur & Desdouets 2010, Hixon & Gualberto 2003), and human fibroblasts and endothelial cells become tetraploid with in vitro replicative aging (Wagner et al. 2001, Walen 2006; T. Davoli & T. de Lange, unpublished data). An increase in polyploidy is also observed in cardiomyocytes with cardiac overloading, congenital heart disease, and hypertension (Adler & Costabel 1975, Staiger et al. 1975) and in hepatocytes with oxidative stress or telomere damage (Gorla et al. 2001, Lazzerini Denchi et al. 2006). Furthermore, polyploidization occurs in inflammatory bronchial lesions (Lothschutz et al. 2002) and in wound healing (Oberringer et al. 1999). The mechanisms and consequences of tetraploidization in these pathological settings are largely unknown.

CHROMOSOME INSTABILITY IN HUMAN CANCER

Most human solid tumors are aneuploid. Karyotypic analysis of cancer cell lines has revealed a wide range of chromosome numbers ranging from hypodiploid to hypertetraploid. A substantial fraction of this aneuploidy, in particular the near-diploid chromosome numbers, can be explained by individual chromosome gains and losses owing to defects in the segregation of sister chromatids (**Figure 1**; reviewed in Holland & Cleveland 2009). This type of chromosome instability (CIN) leads to one chromosome gain or loss event in approximately five cell divisions (Lengauer et al. 1997). In rare cases, the cause of CIN has been assigned to defects in the SAC, cohesion, or the regulation of kinetochore-microtubule attachment (see for a review Thompson et al. 2010). Mutations in Adenomatous Polyposis Coli (APC) in colon carcinoma can result in a CIN phenotype (Fodde et al. 2001a, Kaplan et al. 2001), and loss of Rb has been shown to cause aneuploidy and could therefore explain CIN in many human cancers (Coschi et al. 2010, Manning et al. 2010, van Harn et al. 2010). Aneuploidy can also be caused by oncogene-induced replication stress, which has been proposed to occur in many early neoplastic lesions (see for a review Negrini et al. 2010). Finally, cells with supernumerary centrosomes, which are formed through a tetraploidization event or deregulation of the centrosome cycle, will often missegregate chromosomes owing to transient formation of multipolar spindles (Ganem et al. 2009).

An important but underexplored cause of CIN in the early stages of human cancer is telomere dysfunction (reviewed in de Lange 2005). Short telomeres are frequent in cancer, probably owing to extensive telomere shortening before telomerase is activated. One or more dysfunctional telomeres can be a source of chromosome missegregation because the fusion of

Aneuploidy: state of having a chromosome number different than the diploid one (different than an integer multiple, as in polyploidy)

Adenomatous Polyposis Coli (APC): a tumor suppressor involved in Wnt signaling and frequently mutated in colon cancer

two unprotected chromosome ends can form a dicentric chromosome, which tends to undergo nondisjunction and/or break in mitosis. A unique feature of this source of chromosome instability is that it is episodic, ultimately resulting in aneuploid cancer genomes that stabilize once telomerase has restored telomere function. In this regard, it will be of interest to understand which cancers show ongoing CIN and which have a stable aneuploid chromosome complement.

EVIDENCE FOR TETRAPLOIDIZATION IN HUMAN CANCER

The CIN type of chromosome missegregation does not, however, explain cancers with a triploid or near-tetraploid chromosome number (**Figure 3**; **Table 1**). At the missegregation rate of approximately one per five cell divisions observed in colorectal cancer cell lines, more than 150 population doublings (PD) would be required to generate a clone with close to

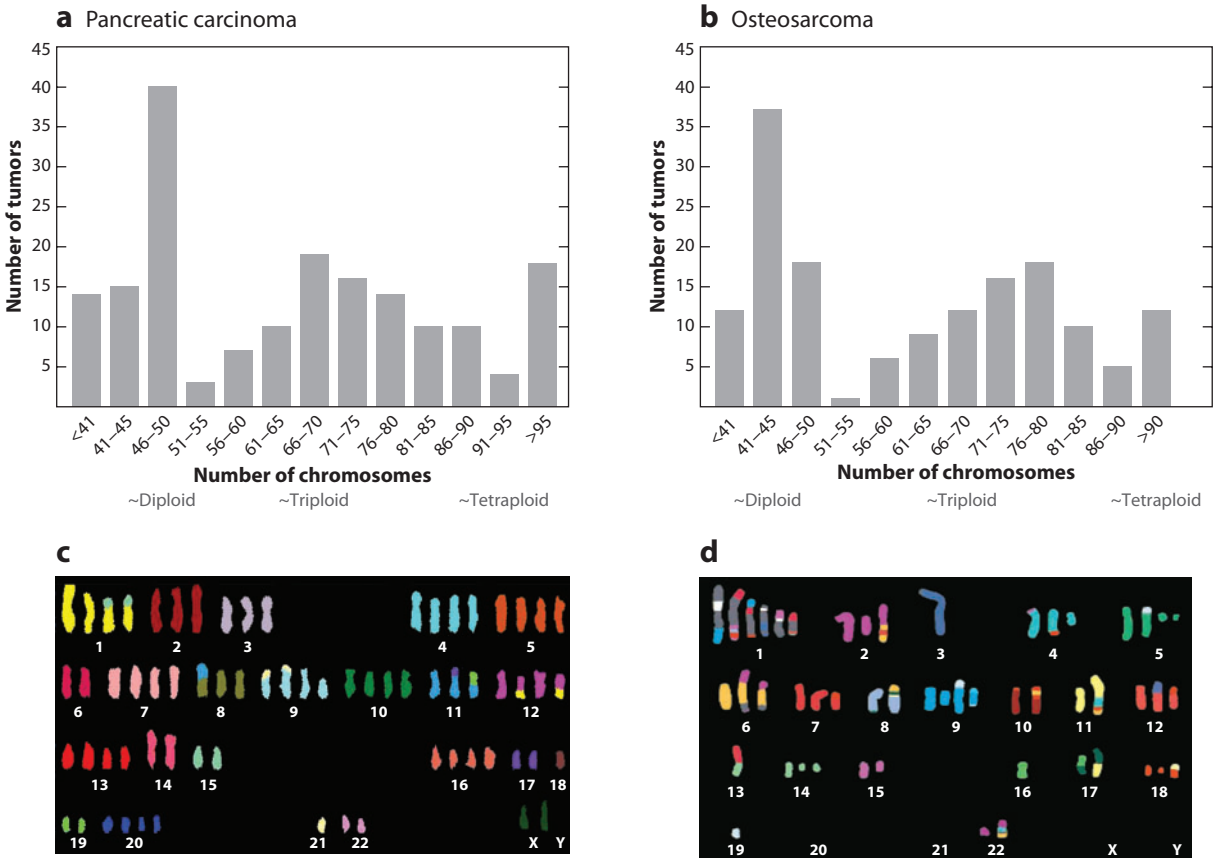

Figure 3

Evidence for tetraploidization in cancer. The bar graphs show the distribution of chromosome numbers in (*a*) pancreatic carcinoma and (*b*) osteosarcoma. The data were derived from the Mitelman database (**http://cgap.nci.nih.gov/Chromosomes/Mitelman**). The karyotypes of all the pancreatic carcinoma and osteosarcoma tumors in the database were retrieved and used to create the frequency distribution shown. For monoclonal tumors, the average number of chromosomes is given (e.g., a tumor with 47–53 chromosomes was scored as having 50 chromosomes). For multiclonal tumors, if the difference between the chromosome numbers of the clones was less than 10, the average is given. Otherwise, the two distinct clones were considered as two different karyotypes. (*c*) Representative karyotype of pancreatic cancer from **http://www.path.cam.ac.uk/~pawefish/index.html** (Capan-2 cell line). (*d*) Representative karyotype of an osteosarcoma tumor from Scheel et al. (2001). Abbreviations: Cdk, cyclin-dependent kinase; Cyc, cyclin.

Table 1 Incidence of hypertriploid karyotypes as well as inactivation of the p53 and Rb pathways

Tumor type (*n*)	Tumors with >68 chromosomes[a]	Inactivation of the p53 pathway[b]	Inactivation of the Rb pathway[c]	References
Liver adenocarcinoma (26)	54%	30–60%	40–60%	Martin & Dufour 2008
Osteosarcoma (143)	42%	40–60%	60–70%	Sandberg & Bridge 2003
Pancreatic adenocarcinoma (140)	41%	50–75%	80–95%	Maitra & Hruban 2008
Lung adenocarcinoma (160)	36%	40–60%	50–80%	Sekido et al. 2003
Cervical carcinoma (60)	34%	>90%[d]	>90%[d]	Moody & Laimins 2010
Neuroblastoma (286)	31%	10–30%[e]	5–20%[e]	Brodeur 2003; Van Maerken et al. 2009
Hodgkin's lymphoma (247)	31%	20–50%	10–30%	Bai et al. 2005; Kuppers 2009
Prostate adenocarcinoma (220)	28%	20–30%	30–50%	Abate-Shen & Shen 2000
Skin squamous cell carcinoma (32)	28%	60–80%	30–50%	Pons & Quintanilla 2006
Soft tissue sarcoma (35)	25%	30–50%	30–60%	Leach et al. 1993; Polsky et al. 2006
Colon adenocarcinoma (345)	24%	60–70%	20–30%	Fearon 2010; Ruas & Peters 1998
Ovarian adenocarcinoma (445)	23%	50–70%	30–50%	Bast et al. 2009
Testis seminoma or teratoma (230)	23%	10–25%	30–60%	Bartkova et al. 2003
Astrocytoma grade III-IV (545)	22%	30–40%	30–50%	Gladson et al. 2010
Esophageal adenocarcinoma (26)	19%	40–50%	40–60%	Maley 2007; Wong et al. 2001
Bladder transitional cell carcinoma (160)	17%	30–50%	50–70%	Mitra & Cote 2009
Breast adenocarcinoma (772)	17%	20–30%	40–50%	Shackney & Silverman 2003
Kidney carcinoma (1238)	13%	10–30%	10–30%	Aaltomaa et al. 1999; Lipponen et al. 1995; Noon et al. 2010
Malignant melanoma (350)	13%	10–20%	30–50%	Ibrahim & Haluska 2009
Stomach carcinoma (115)	11%	30–40%	20–30%	Wu et al. 2010

[a]Based on **http://cgap.nci.nih.gov/Chromosomes/Mitelman**. For each tumor type, a search of the tumors containing a chromosome number >68 was performed, and the percentage of such tumors was calculated. The total number of tumors for each type is indicated (*n*).
[b]Frequency of inactivation of the p53 pathway, including p53 mutation (databases **http://www-p53.iarc.fr/**, **http://p53.free.fr/index.html**, and specific references) or loss of heterozygosity (LOH) and amplification of murine double minute 2 (MDM2) (Momand et al. 1998).
[c]Frequency of inactivation of the Rb pathway, including mutation; LOH; or methylation of Rb, p16, p18 and amplification or overexpression of CyclinD or CDK4/6 genes (Ruas & Peters 1998, Sharpless & Chin 2003).
[d]In cervical cancer, p53 and Rb inactivation are mediated by HPV-E6 and HPV-E7 proteins, respectively (Moody & Laimins 2010).
[e]The frequency of inactivation of the p53 and Rb pathways in neuroblastoma is not completely clear because most studies have been performed on cell lines (Brodeur 2003, Van Maerken et al. 2009).

75 chromosomes. This number of cell divisions is quite high in the context of the etiology of human cancer. In addition, each intermediate in the multistep CIN pathway would have to be viable.

Data on the distribution of chromosome numbers in human cancer are consistent with the proposal of a second, distinct pathway for tumors with high chromosome numbers. For many tumors, the chromosome numbers are distributed into two peaks, one representing tumors that are near-diploid and one representing tumors with a chromosome number between a triploid and a tetraploid genome (Storchova & Kuffer 2008) (see examples in **Figure 3**). This bimodal distribution argues against a single mechanism underlying aneuploidy. To account for aneuploidy with high chromosome numbers (arbitrarily set at >68 chromosomes in **Table 1**), Shackney et al. (1989) proposed that these cancers originate from an unstable tetraploid intermediate. As discussed above, tetraploid cells are known to frequently missegregate chromosomes

Barrett's esophagus: precancerous lesion that predisposes to esophageal adenocarcinoma

owing to their supernumerary centrosomes (Ganem et al. 2009). Tetraploid cells will therefore readily generate subclones with the hypotetraploid or hypertriploid chromosome numbers observed in cancer.

Consistent with an initial tetraploidization event preceding the final aneuploid state, cells with supernumerary centrosomes have been observed in many tumor types, including breast cancer (Lingle et al. 1998), pancreatic cancer (Sato et al. 1999), prostate cancer (Pihan et al. 2001), and lung and colon carcinoma (Pihan et al. 1998). Multiple centrosomes appear in preinvasive carcinomas (Lingle et al. 2002, Pihan et al. 2003) and correlate with abnormal multipolar mitosis and chromosomal instability (Lingle et al. 2005).

In some tumor types, there is direct evidence for the development of aneuploidy from a transient 4N state. In Barrett's esophagus, which predisposes to the development of esophageal adenocarcinoma, tetraploid cells precede the development of further aneuploidy (Galipeau et al. 1996, Reid et al. 1996). The tetraploid state is lost in 1–2 years, consistent with transient tetraploid cells that lose chromosomes at high frequency (Galipeau et al. 1996). Similarly, during the evolution of cervical, breast, and bladder cancer, there is evidence for whole-genome duplication followed by chromosome losses (Dutrillaux et al. 1991; Kirkland et al. 1967; Olaharski et al. 2006; Ottesen 2003; Shackney et al. 1995a,b). Tetraploidization also occurs in hyperplastic lesions of the pancreas (Tanaka et al. 1984), in localized prostate cancer (Deitch et al. 1993, Montgomery et al. 1990, Pihan et al. 2001), and some colon adenomas (Hamada et al. 1988, Levine et al. 1991). Collectively, these observations argue that tetraploidization in the early stages of tumorigenesis is not a rare phenomenon.

CAUSES OF TETRAPLOIDY IN CANCER

Three distinct causes of tetraploidization in cancer have been proposed: cell fusion, failure in cytokinesis or other steps in mitosis, and endoreduplication (**Figure 4**). Cell fusion generates a binucleate intermediate that can produce daughter cells with single 4N nuclei in G1. Experimentally induced fusion of primary human fibroblasts has been shown to enhance their in vitro transformation with potent oncogenes (Duelli et al. 2007). Viral infection can cause cell fusion (see for a review Duelli & Lazebnik 2007). For instance, infection with human papilloma virus (HPV), which contributes to the etiology of cervical cancer, has been shown to cause cell fusion (Gao & Zheng 2010, Hu et al. 2009). The virus that causes cell fusion also could be unrelated to cancer development because the fusion partner could be a nonneoplastic infected cell that donates its chromosomes to a cancerous neighbor.

Several types of failure in progression through, or exit from, mitosis can give rise to a cell with double the chromosome number. Failure in cytokinesis usually generates a transient binucleate state that will yield mononucleated G1 daughter cells with 4N DNA content after the next cell division (**Figure 4**). This type of tetraploidization is observed upon increased expression of either of two regulators of the anaphase-promoting complex, mitotic arrest deficient 2 (Mad2) or Emi1 (Lehman et al. 2006, Sotillo et al. 2007). Mad2 and Emi1 are overexpressed in a variety of human cancers (Lehman et al. 2007, Sotillo et al. 2007), possibly owing to loss of the Rb pathway, which controls their transcription through E2F. Furthermore, Mad2 overexpression might induce tetraploidization through inactivation of MLKP2, a kinesin required for cytokinesis (Lee et al. 2010). Cytokinesis failure also occurs upon inhibition of large tumor suppressor 1 (LATS1), a kinase needed for the regulation of actin polymerization in mitosis (Yang et al. 2004). LATS1 is a tumor suppressor in *Drosophila* and is frequently lost in soft tissue sarcoma (Hisaoka et al. 2002), astrocytoma (Jiang et al. 2006), and breast cancer (Takahashi et al. 2005). Finally, failure in cytokinesis is associated with overexpression of AuroraA, a kinase critical for mitosis (Bischoff et al. 1998; Meraldi et al. 2002;

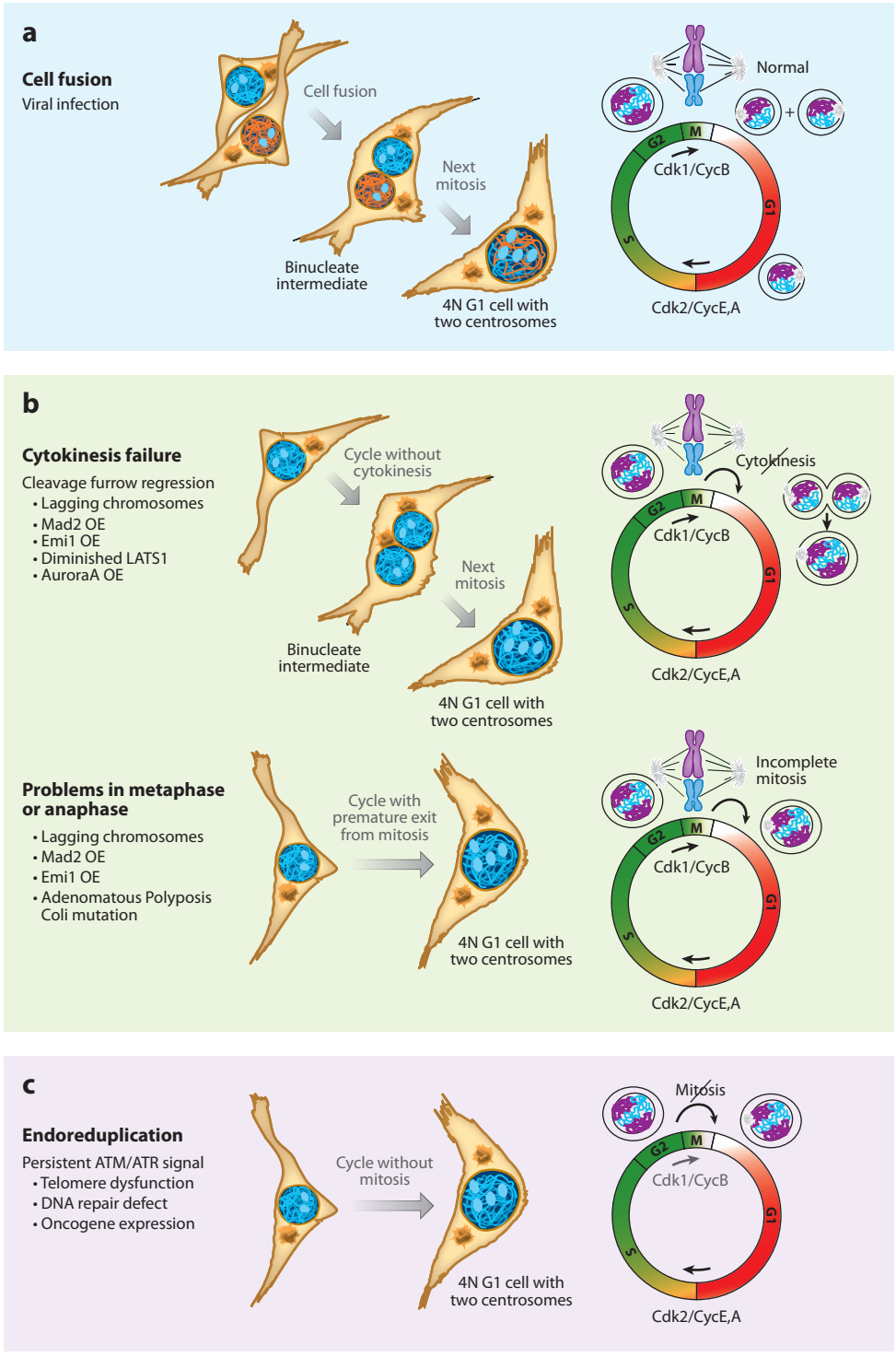

Figure 4

Proposed mechanisms for tetraploidization in cancer. The schematic shows the three main mechanisms by which tetraploidization has been proposed to arise in the early stages of tumorigenesis: (*a*) cell fusion, (*b*) cytokinesis failure or problems in metaphase or anaphase, and (*c*) endoreduplication. Abbreviations: ATM/ATR, ataxia telangiectasia mutated/ATM and Rad3-related; Cdk, cyclin-dependent kinase; Cyc, cyclin; LATS1, large tumor suppressor 1; Mad2, mitotic arrest deficient 2; OE, overexpression.

AuroraA: Ser/Thr protein kinase necessary for proper formation and function of the mitotic spindle

Emi1: cell cycle–regulated F-box protein that negatively regulates the activity of APC/C

Large tumor suppressor 1 (LATS): a Ser/Thr kinase involved in mitosis and other cellular processes that is frequently mutated in cancer

Mitotic arrest deficient 2 (Mad2): a negative regulator of APC/Cdc20 required for the spindle assembly checkpoint

Zhang et al. 2004, Zhou et al. 1998). High levels of AuroraA, in some cases due to gene amplification, are frequent in cancer (Lehman et al. 2007). In light of these potential causes of cytokinesis failure, it will be of interest to determine whether evidence supports an initial tetraploidization step in tumors with elevated Mad2/Emi1/AuroraA or defective LATS1.

Cells with lagging chromosomes or acentric fragments can experience failure in cytokinesis because the cleavage furrow can regress when chromatin persists in the midzone (Mullins & Biesele 1977). Because a myriad of pathways can result in lagging chromosomes or acentric fragments, many genetic defects potentially could result in a low frequency of tetraploidization through this mechanism.

Problems earlier in mitosis are also likely to be responsible for some tetraploidization events. Interestingly, tetraploidization occurs in cells with defects in Adenomatous Polyposis Coli (APC), the colon carcinoma gene that is part of the Wnt signaling pathway (Fodde et al. 2001a, Kaplan et al. 2001). This APC protein (which is distinct from the APC/C) binds to microtubules, and its absence affects the kinetochore-microtubule interaction in a manner that induces the disordered metaphases, lagging chromosomes, and chromosome missegregation responsible for CIN in APC-driven colon carcinoma (Caldwell et al. 2007; Dikovskaya et al. 2004, 2007; Draviam et al. 2006; Fodde et al. 2001a; Kaplan et al. 2001). The function of the SAC is not greatly affected by these APC mutations (Radulescu et al. 2010), nor is the SAC strongly activated by the disordered metaphases in APC-mutant cells (Draviam et al. 2006). Whether tetraploidization of APC-mutant cells is a direct consequence of the lagging chromosomes is not yet clear.

Recently, we found that a persistent DNA damage response can also induce tetraploidy (Davoli et al. 2010; **Figure 4**). Activation of the DNA damage checkpoint in p53- and Rb-deficient cells normally results in a G2/M arrest that is relieved once the integrity of the genome is reestablished. However, in cells with irreparable damage or cells that experience repeated genotoxic insults, the G2/M arrest ultimately acquiesces. These cells lack Cdk1/CycB activity and therefore do not progress into mitosis. Rather, they skip mitosis, enter a G1-like state, and progress into a second S phase. After a prolonged G2 arrest, the cells exhibit mitosis-independent degradation of geminin, which allows Cdt1 to relicense replication origins. Geminin degradation is dependent on APC/Cdh1 in these endocycles, just as it is in a normal cell cycle. Because Cdk1/CycB inhibits APC/Cdh1 (Zachariae et al. 1998), the absence of mitotic Cdk activity is likely to create a permissive setting for APC/Cdh1 activation. As in the case of placental trophoblast polyploidization (**Figure 2**), the duplicated chromatids can remain linked because APC/Cdc20 is not activated, resulting in diplochromosomes during the next mitosis (Davoli et al. 2010).

Tetraploidization arises when the DNA damage signal lasts for a considerable time period, a situation that can be created by incubating cells continuously with zeocin or doxorubicin so that DNA damage is unremitting despite ongoing repair. The most likely physiological source of such persistent DNA damage is the loss of telomere function early in tumorigenesis. Owing to the absence of telomerase activity, proliferating human somatic cells undergo progressive telomere shortening (**Figure 5**; see for a review Shay & Wright 2005). Short telomeres are recognized as sites of DNA damage, activating ATM/ATR and Chk1/Chk2, which results in a p53/Rb-mediated permanent arrest. In the absence of the p53 and p16/Rb pathways, extended proliferation beyond the senescence limit exacerbates telomere shortening resulting in a stage, called crisis, that is characterized by massive chromosomal instability (**Figure 5**). Telomere shortening is observed in the initial stages of epithelial cancers (Meeker et al. 2004), and the short telomeres of most human solid tumors bear witness to this process (De Lange 2005). Eventually, activation of telomerase and restoration of telomere function can counteract this tumor suppressor mechanism.

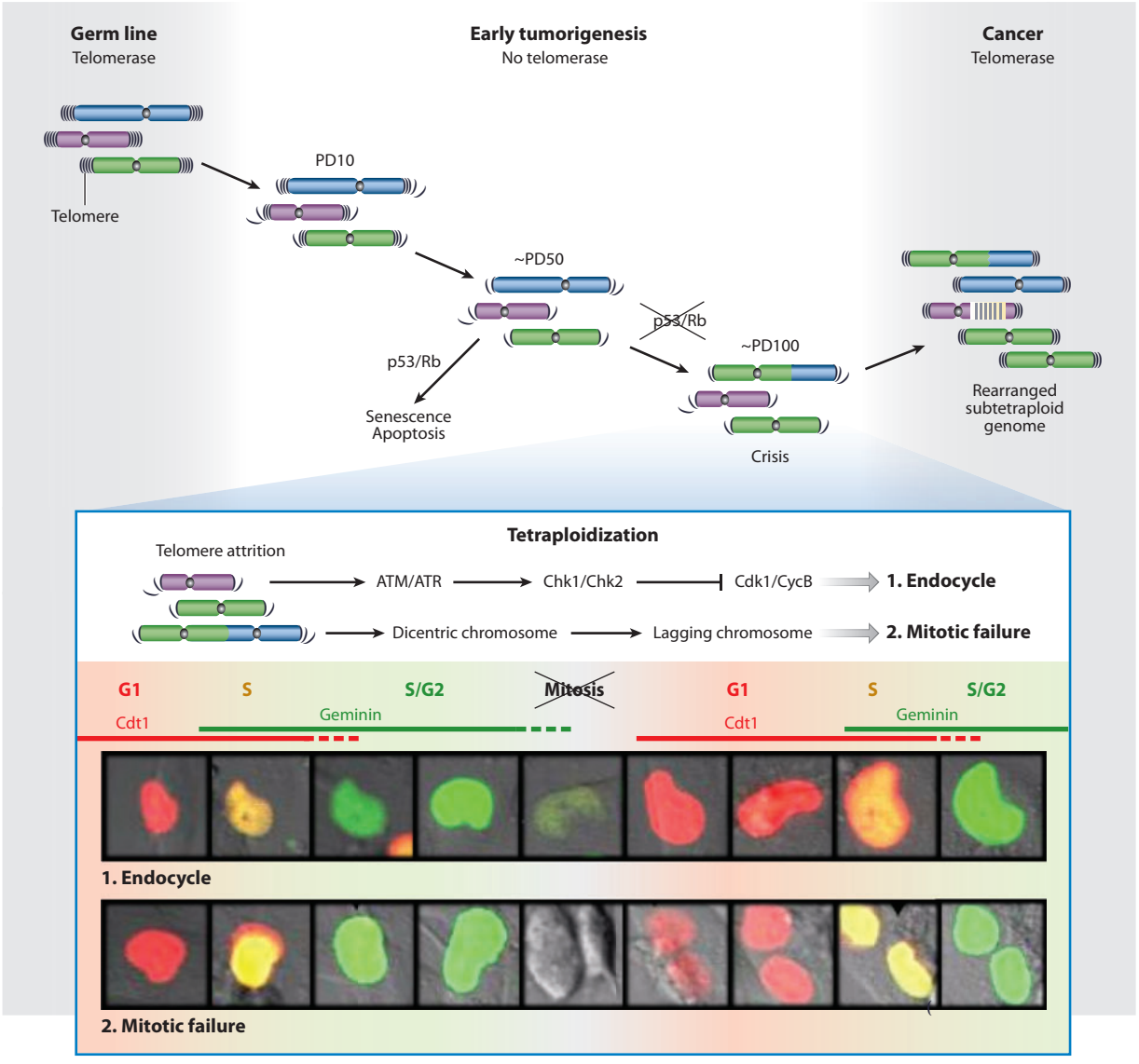

Figure 5

Consequences of telomere dysfunction in cancer development. The figure illustrates the process of telomere shortening in human somatic cells and its consequences for the development of an aneuploid tumor karyotype. Extensive telomere attrition in the absence of p53/Rb pathways to the state of crisis can lead to tetraploidy through endoreduplication or mitotic failure. Photographs from live-cell imaging [fluorescent ubiquitination-based cell cycle indicator (FUCCI) system; Sakaue-Sawano et al. (2008)] of human cells in crisis undergoing endoreduplication or mitotic failure are shown (T. Davoli & T. de Lange, submitted). The endocycle, resulting from persistent telomere dysfunction, is characterized by alternating oscillations of Cdt1 (G1/early S) and geminin (S/G2) and by mitosis-independent geminin degradation. Mitotic failure, owing to the presence of dicentric chromosomes, involves normal entry into mitosis and geminin degradation during anaphase. However, cytokinesis is not completed, resulting in a binucleated cell. In cancer, telomerase reactivation may allow proliferation of the tetraploid cells by reestablishing telomere protection and silencing the DNA damage response signal emanating from telomeres. Abbreviations: ATM/ATR, ataxia telangiectasia mutated/ATM and Rad3-related; Cdk, cyclin-dependent kinase; Chk, checkpoint kinase; Cyc, cyclin; PD, population doubling.

***BRCA2*:** a breast cancer susceptibility gene encoding a protein involved in DNA repair

Recent data indicate that the telomere dysfunction during crisis is a likely instigator of tetraploidization in cancer. When telomeres become dysfunctional owing to shelterin inactivation (Davoli et al. 2010) or as a consequence of extensive telomere shortening (T. Davoli & T. de Lange, submitted), tetraploidization occurs frequently. The tetraploidization can be induced by persistent DNA damage signaling through endoreduplication, exhibiting the characteristic mitosis-independent degradation of geminin (**Figure 5**). In addition to endoreduplication, tetraploidization in crisis can result from failure in mitosis owing to the presence of dicentric chromosomes. If the two centromeres of a dicentric chromosome are pulled toward opposite poles of the mitotic spindle, the resulting lagging chromosome can prevent completion of mitosis (**Figure 5**). The resulting tetraploid cells can proliferate after restoration of their telomeres (Davoli et al. 2010). Therefore, postcrisis healing of telomeres by telomerase should allow expansion of a tetraploid clone in the early stages of tumorigenesis.

In addition, a persistent DNA damage signal might induce endoreduplication in two other situations (**Figure 4**). The first is a setting in which homology-directed repair (HDR) is impaired. HDR is an essential pathway required for the repair of spontaneous damage in S phase. When HDR is diminished, unresolved S phase problems could elicit a persistent DNA damage signal in G2 and induce tetraploidization. XRCC3-deficient cells, which have impaired HDR, have a low frequency of endoreduplication (Yoshihara et al. 2004). Furthermore, overexpression of RPA, or deletion of Nbs1 and Rad17, results in endoreduplication, most likely because of unrepaired DNA damage (Reina-San-Martin et al. 2005, Wang et al. 2003). The relevance of these observations to tetraploidization in human cancer remains to be determined, but notably, p53-deficient breast cancers lacking the HDR protein BRCA2 are often polyploid (Gretarsdottir et al. 1998).

A second cancer-relevant setting in which a persistent DNA damage response signal could cause tetraploidization is replication stress induced by certain oncogenes. Activation of the DNA damage response, presumably owing to unscheduled DNA replication, is frequently observed in hyperproliferative preneoplastic lesions, and a DNA damage response is induced upon oncogene expression in cultured cells (Bartkova et al. 2005, 2006; Di Micco et al. 2006; Gorgoulis et al. 2005). Furthermore, prolonged oncogene expression can induce tetraploidization in U2OS osteosarcoma cells and IMR90 fibroblasts (Bartkova et al. 2006, Chicas et al. 2010), perhaps as a consequence of a persistent DNA damage response.

ROLE OF P53 IN CULLING TETRAPLOID CLONES

Tetraploid cells generated by experimentally induced mitotic failure undergo a p53-dependent arrest (Andreassen et al. 2001, Fujiwara et al. 2005, Meraldi et al. 2002). This activation of the p53 pathway is also thought to occur in other cases of tetraploidization, but the mechanism underlying this phenomenon is not understood. The p53 pathway is unlikely to be activated by tetraploidy per se or by the presence of extra centrosomes (Uetake & Sluder 2004, Wong & Stearns 2005). Although the signal(s) that upregulate p53 remain to be determined, the challenges of navigating mitosis with supernumerary centrosomes make tetraploid cells more prone to sustaining genome instability, which may explain why they proliferate better in the absence of p53 (Ganem et al. 2009). In agreement with this, the frequency of p53 pathway inactivation and the occurrence of near-tetraploid karyotypes in cancer are positively correlated (**Figure 6*a***). In the case of telomere dysfunction and Emi1- or Mad2-induced tetraploidy, inactivation of the Rb pathway is expected to be a second critical factor. Interestingly, there is a positive correlation between the occurrence of near-tetraploid karyotypes and the frequency of inactivation of the Rb pathway (**Figure 6*b***), which suggests an important (but unexpected) role for Rb loss in tetraploidization during tumorigenesis.

CONSEQUENCES OF TETRAPLOIDY IN CANCER

The challenge for the incipient cancer cell is to evolve the right combination of genetic alterations that support unbridled proliferation at inappropriate locations within the body. Aneuploidy per se does not confer an advantage in this regard, but the reassortment of mutated alleles in the process leading to aneuploidy does. Tetraploidization and its associated aneuploidy are particularly well-suited to accelerate tumor genome evolution for two reasons. First, tetraploidy is likely to enhance robustness in the face of a mutator phenotype, as it will buffer the consequences of chromosome losses, gene deletions, and inactivating mutations. Thompson et al. (2006) tested this idea directly by comparing diploid and haploid yeast, and it is likely to hold for diploid versus tetraploid mammalian cells in which imprinting and haploinsufficiency render part of the genome functionally haploid. Thus, tetraploidy is expected to allow tumor cells to sustain a higher incidence of mutations, thereby increasing the probability of adaptive changes. Second, tetraploid cells have an increased rate of chromosome missegregation (Mayer & Aguilera 1990, Storchova et al. 2006) as a consequence of supernumerary centrosomes (Ganem et al. 2009). Thus, tetraploidy will increase the probability that an evolving tumorigenic clone will accumulate and tolerate the mutations needed for its progression to a malignant state.

Despite these selective advantages, the problems associated with supernumerary centrosomes limit the survival and proliferation of tetraploid cells. Cells can negotiate these problems through elimination/inactivation of centrosomes or by clustering them into two groups so that a bipolar spindle is formed (reviewed in Godinho et al. 2009). Centrosome clustering appears to be the dominant route by which (sub)tetraploid cancer cells avoid multipolar spindles, and factors required for this clustering are potential targets for cancer therapy (Kwon et al. 2008).

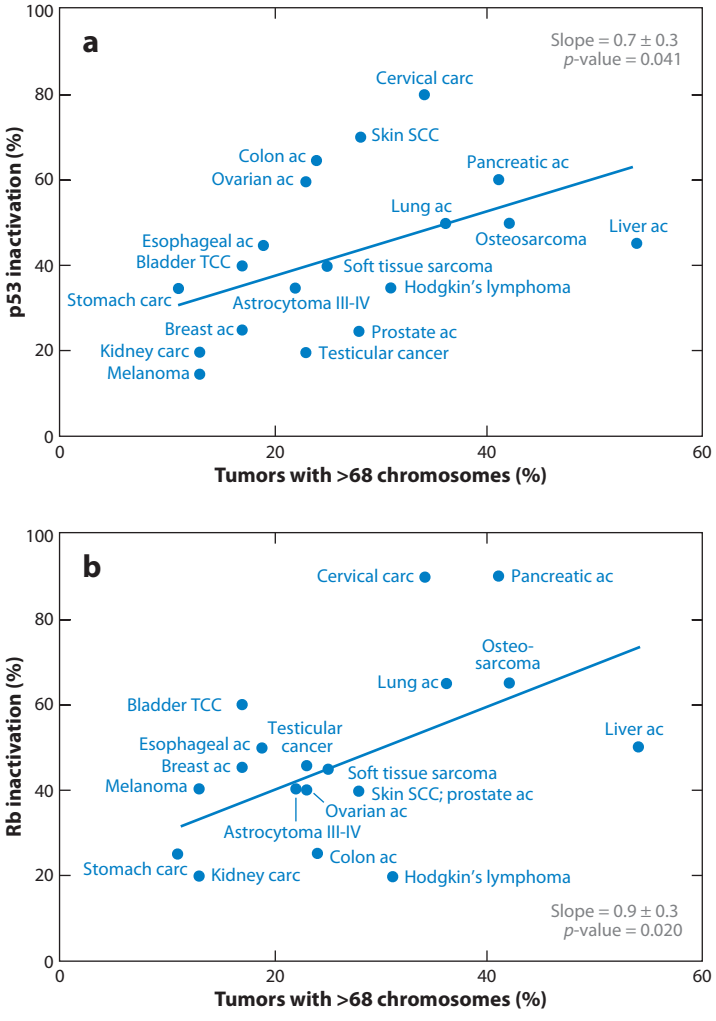

Figure 6

Correlation between the frequency of inactivation of the (*a*) p53 and (*b*) Rb pathways and the percentage of karyotypes with high chromosome number (hypertriploid karyotypes; data from **Table 1**) for the indicated cancers. Linear regression analysis indicates significant correlation (slope and p-value are reported from the F-test using Prism-5 software). Abbreviations: ac, adenocarcinoma; carc, carcinoma; SCC, squamous cell carcinoma; TCC, transitional cell carcinoma.

TIMING OF TETRAPLOIDIZATION DURING TUMORIGENESIS

The outgrowth of all tetraploid clones is blocked by the p53 pathway and, in the case of DNA damage-induced tetraploidization, also by the p16/Rb pathway. Therefore,

Ductal carcinoma in situ (DCIS): the most common form of noninvasive breast carcinoma

tetraploidization is more likely to contribute to those cancers in which the relevant tumor suppressor pathways are inactivated frequently at an early stage, before the emergence of the tetraploidization stimulus. Although much of the relevant information is missing, it is important to consider the potential order of events in cancer types in which tetraploidization is frequent (**Figure 7**).

In the case of virally induced cell fusion, the presumed causative agent is elusive. The exception is HPV-induced cervical cancer, in which viral infection could both induce fusion and, through the action of HPV E6, inactivate p53 (Moody & Laimins 2010), thus creating a permissive setting for the proliferation of tetraploid cells (**Figure 7**).

Tetraploidization induced by the failure in progression through mitosis is a more complex scenario because mitotic failure can be induced in a variety of ways (**Figure 4**). In the case of APC mutations in colon carcinoma, p53 loss is a later event (Fodde et al. 2001b), suggesting that mitotic failure might take place early but will result in viable tetraploid clones only at a later stage. Similarly, for Mad2 and Emi1 overexpression owing to inactivation of the Rb pathway, the mitotic problems induced by Mad2 and Emi1 are unlikely to give rise to tetraploid clones unless p53 is inactivated. For instance, in lung adenocarcinoma, inactivation of Rb precedes the loss of p53, which occurs at the time that invasive cancer develops (Noguchi 2010; **Figure 7**). Therefore, tetraploid clones induced by overexpression of Mad2 or Emi1 are most likely to arise at the transition to invasive cancer (Garber et al. 2001, Lehman et al. 2007). In breast cancer, inactivation of Rb and p53 is observed relatively early, allowing Mad2- or Emi1-driven tetraploidization events to contribute to tumor progression at an early invasive stage (Lehman et al. 2007, Shackney & Silverman 2003, van 't Veer et al. 2002; **Figure 7**). Other sources of cytokinesis failure such as inhibition of LATS1 or AuroraA overexpression are more difficult to evaluate because the timing of these changes is not known. An exceptional case is liver adenocarcinoma. Because hepatocytes are prone to undergo tetraploidization despite a functional p53 pathway (**Figure 2**), Mad2/Emi1-induced tetraploidization may well occur at an early stage when Rb is inactivated but p53 is not (Hui et al. 1998, Martin & Dufour 2008, Teramoto et al. 1994; **Figure 7**).

The third potential cause of tetraploidization, endoreduplication or mitotic failure owing to dysfunctional telomeres, requires the correct order of three events. First, the p53 and p16/Rb pathways need to be disabled to allow cells to proliferate to the point of telomere crisis. Second, tetraploidization needs to occur. Third, telomerase must be activated to restore telomere function and avoid further telomere shortening. Telomere shortening is notable in the preinvasive stages of most epithelial cancers (Meeker & Argani 2004; Meeker et al. 2002, 2004; van Heek et al. 2002), which is also when inactivation of the p53 and Rb pathways occurs in many tumors (**Figure 7**). For instance, in breast cancer, tetraploidization occurs at the ductal carcinoma in situ (DCIS) stage when the p53 and Rb pathways are usually inactivated and telomerase is increasingly detectable

Figure 7

Timing of loss of p53/Rb, activation of telomerase, and tetraploidization in select human cancers. For a subset of cancers with evidence for tetraploidization, the potential causes of tetraploidy, the incidence of karyotypes with high chromosome numbers (hypertriploid karyotypes, from **Table 1**), and the timing of the loss of p53 and Rb pathways (% from **Table 1**), activation of telomerase, and tetraploidization are indicated. Four stages of neoplastic progression are considered: dysplasia, in situ cancer lesion, invasive cancer, and advanced/metastatic cancer. Evidence for tetraploidization includes data on chromosome number in metaphase spreads, fluorescence-activated cell sorting analysis of the DNA content, and supernumerary centrosomes. Abbreviations: APC, Adenomatous Polyposis Coli; downreg, downregulation; HPV, human papilloma virus; LATS1, large tumor suppressor 1; Mad2, mitotic arrest deficient 2; mut, mutation; OE, overexpression.

Tumor type	Potential causes of tetraploidy	Incidence and sequence of events: Dysplasia / Cancer in situ / Invasive cancer / Advanced/metastatic	Hyper-triploid tumors
Liver adenocarcinoma	Mad2 OE (80%)[53] Emi1 OE (80%)[19] AuroraA OE (50%)[19] Telomere shortening[42]	Tetraploidy[4] p53 inactive (30–60%)[26,50] Rb inactive (40–60%)[16,26] Telomerase active[42]	54%
Pancreatic adenocarcinoma	AuroraA OE[19] Telomere shortening[52]	Tetraploidy[49,41] p53 inactive (50–75%)[24] Rb inactive (80–95%)[24] Telomerase active[24,47]	41%
Lung adenocarcinoma	Mad2 OE[12] Emi1 OE (65%)[19] Telomere shortening[9]	Tetraploidy[23] p53 inactive (40–60%)[36] Rb inactive (50–80%)[36] Telomerase active[9]	36%
Cervical carcinoma	HPV infection (>90%)[34] Emi1 OE (80%)[19] AuroraA OE (50%)[19] Telomere shortening[28]	Tetraploidy[18,37] p53 inactive (>90%)[34] Rb inactive (>90%)[34] Telomerase active[13]	34%
Prostate adenocarcinoma	Telomere shortening[27]	Tetraploidy[5,33] p53 inactive (20–30%)[1] Rb inactive (30–50%)[1] Telomerase active[29]	28%
Colon adenocarcinoma	APC mut (50–70%)[40] Mad2 OE[10] AuroraA OE (60%)[19] Telomere shortening[26]	Tetraploidy[14,20] p53 inactive (60–70%)[8] Rb inactive (20–30%)[8] Telomerase active[7,46]	24%
Ovarian adenocarcinoma	Emi1 OE (60–100%)[19] AuroraA OE (60%)[19] Telomere shortening[35]	Tetraploidy[3,17] p53 inactive (50–70%)[2] Rb inactive (30–50%)[2] Telomerase active[35]	23%
Esophageal adenocarcinoma	Telomere shortening[28]	Tetraploidy[11,37] p53 inactive (40–50%)[25] Rb inactive (40–60%)[25] Telomerase active[25]	19%
Breast adenocarcinoma	Mad2 OE[51] Emi1 OE (45%)[19] AuroraA OE[32] LATS1 downreg.[48] Telomere shortening[30]	Tetraploidy[6,38,43] p53 inactive (20–30%)[6,45,43] Rb inactive (40–50%)[45] Telomerase active[15,46]	17%
Bladder transitional cell carcinoma	Emi1 OE (45%)[19] Telomere shortening[28]	Tetraploidy[44] p53 inactive (30–50%)[31] Rb inactive (40–70%)[31] Telomerase active[22]	17%

[1]Abate-Shen & Shen 2000
[2]Bast et al. 2009
[3]Braly & Klevecz 1993
[4]Celton-Morizur & Desdouets 2010
[5]Deitch et al. 1993
[6]Dutrillaux et al. 1991
[7]Engelhardt et al. 1997
[8]Fearon 2010
[9]Fernandez-Garcia et al. 2008
[10]Friederichs et al. 2005
[11]Galipeau et al. 1996
[12]Garber et al. 2001
[13]Gupta et al. 2010
[14]Hamada et al. 1988
[15]Herbert et al. 2001
[16]Hui et al. 1998
[17]Kallioniemi et al. 1988
[18]Kirkland et al. 1967
[19]Lehman et al. 2007
[20]Levine et al. 1991
[21]Li et al. 2003
[22]Lin et al. 1996
[23]Lothschutz et al. 2002
[24]Maitra & Hruban 2008
[25]Maley 2007
[26]Martin & Dufour 2008
[27]Meeker et al. 2002
[28]Meeker et al. 2004
[29]Meeker 2006
[30]Meeker & Argani 2004
[31]Mitra & Cote 2009
[32]Miyoshi et al. 2001
[33]Montgomery et al. 1990
[34]Moody & Laimins 2010
[35]Murakami et al. 1997
[36]Noguchi 2010
[37]Olaharski et al. 2006
[38]Ottesen, 2003
[39]Reid et al. 1996
[40]Samowitz et al. 2007
[41]Sato et al. 1999
[42]Satyanarayana et al. 2004
[43]Shackney et al. 1995
[44]Shackney et al. 2003
[45]Shackney & Silverman 2003
[46]Shay & Bacchetti 1997
[47]Suehara et al. 1997
[48]Takahashi et al. 2005
[49]Tanaka et al. 1984
[50]Teramoto et al. 1994
[51]van 't Veer et al. 2002
[52]van Heek et al. 2002
[53]Zhang et al. 2008

(Herbert et al. 2001, Ottesen 2003, Shackney & Silverman 2003, Shay & Bacchetti 1997). In addition, there is good evidence for telomere crisis in DCIS followed by stabilization of the genome owing to telomerase-mediated telomere restoration (Chin et al. 2004). Similarly, in Barrett's esophagus, Rb inactivation is an early event that is followed by p53 dysfunction and telomerase activation at the preinvasive stage when tetraploidy arises (Maley 2007, Reid et al. 1996). In pancreatic adenocarcinoma, frequent inactivation of the Rb and p53 pathways also takes place before the activation of telomerase at an early stage (usually in intraepithelial/in situ neoplasia) when tetraploidy is observed (Maitra & Hruban 2008, Sato et al. 1999, Suehara et al. 1997, Tanaka et al. 1984, van Heek et al. 2002). In bladder, ovarian, and prostate cancer, inactivation of the Rb/p53 pathways and telomerase reactivation occur at approximately the same time, at the transition from in situ to invasive cancer, when there is evidence of tetraploidy (Abate-Shen & Shen 2000, Bast et al. 2009, Braly & Klevecz 1993, Kallioniemi et al. 1988, Lin et al. 1996, Meeker 2006, Mitra & Cote 2009, Montgomery et al. 1990; Murakami et al. 1997, Shackney et al. 1995b). In sum, in several tumor types with evidence for tetraploidization (**Figure 7**), the order of p53/Rb inactivation, occurrence of tetraploidy, and telomerase upregulation are consistent with telomere dysfunction as the stimulus for tetraploidization. This contrasts with other cancers in which the correct order of events is unlikely to occur. For instance, in kidney carcinoma, inactivation of the p53 and Rb pathways is rare and occurs late, often in advanced or metastatic tumors (Ibrahim & Haluska 2009). Indeed, the near-diploid karyotype of most kidney carcinomas argues against tetraploidization in the formation of this cancer type. Similarly, tetraploidization is rare in human tumors arising from telomerase-competent cells in which telomerase activation presumably precedes other events. For example, near-diploid karyotypes are present in most human leukemias and some lymphomas, which originate from telomerase-competent cells (Broccoli et al. 1995, Hilgenfeld et al. 1999).

FUTURE PERSPECTIVES

The data summarized here argue that inappropriate tetraploidization is a frequent event in the early stages of cancer development. Insight into this important and pervasive pathway toward aneuploidy can potentially illuminate the nature of genome instability in cancer and may reveal cancer-specific vulnerabilities that can be exploited in the clinic. Progression through a tetraploid intermediate likely requires adaptations that may become permanent in the developing cancer lineage. Such adaptations, which include but are not limited to the clustering of centrosomes to allow bipolar spindle formation, may well be targetable and should be cancer specific. In this regard, it will be important to identify the mechanisms and/or instigators of whole-genome duplication for each cancer type. A deeper knowledge of the causes and consequences of tetraploidization in cancer could also deliver diagnostic tools to parse subsets of cancers on the basis of their route to aneuploidy. Such parsing may be required to optimize cancer treatment on the basis of past tetraploidization.

DISCLOSURE STATEMENT

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