CENTROSOME ABERRATIONS: CAUSE OR CONSEQUENCE OF CANCER PROGRESSION?

Erich A. Nigg

Many human tumours show centrosome aberrations, indicating an underlying deregulation of centrosome structure, duplication or segregation. Centrosomes organize microtubule arrays throughout the cell cycle, thereby influencing both tissue architecture and the accuracy of chromosome segregation. But what are the origins of centrosomal abnormalities in tumours, and what impact do they have on the generation of invasive, genetically unbalanced cells during cancer progression?

CYTOKINESIS
The process of cytoplasmic division.

SPINDLE
A dynamic bipolar array of microtubules that is assembled during mitosis and meiosis to segregate chromosomes.

ASTERS
Radial microtubule arrays with minus ends that are usually tethered to centrosomes (or assemblies of centrosomal proteins) and plus ends that extend towards the periphery.

CLEAVAGE PLANE
The plane of cell division — defined by the assembly of a contractile actomyosin ring at the cell cortex.

Max Planck Institute of Biochemistry, Department of Cell Biology, Am Klopfersitz 18a, D-82152 Martinsried, Germany.
e-mail: nigg@biochem.mpg.de
doi:10.1038/nrg924

Centrosomes are the microtubule-organizing centres of animal cells[1–3]. By controlling the number, polarity and distribution of microtubules, they coordinate all microtubule-related functions. These include cell shape, polarity, adhesion and motility, as well as the intracellular transport and positioning of organelles. Furthermore, centrosome function is crucial for chromosome segregation and CYTOKINESIS. Although certain female germ cells can assemble bipolar SPINDLES in the absence of centrosomes, the number of centrosomes that are present in somatic cells determines the number of spindle poles. Normally, the two centrosomes that are present at the onset of mitosis instruct the formation of a bipolar spindle. Extra copies frequently result in the formation of multipolar spindles, and failure of centrosomes to separate results in monopolar ASTERS. Aberrations in the number of centrosomes therefore almost inevitably cause chromosome missegregation. Centrosomes also determine the positioning of the CLEAVAGE PLANE during cytokinesis, which is essential for asymmetric divisions and morphogenesis.

Considering the multitude of cellular properties that depend on accurate centrosome function, it is not surprising that the structure and number of these organelles is tightly regulated throughout the cell cycle[2,4,5]. Conversely, there is evidence that the centrosome contributes to cell-cycle regulation and checkpoints[3,6–9]. As early as 1914, Theodor Boveri proposed a direct link between centrosomal abnormalities and both the aneuploidy (BOX 1) and loss of tissue architecture that are typical of human tumours[10]. Sparked by the demonstration that centrosomal abnormalities are frequent in many common cancers[11–13], interest in this old hypothesis has staged an impressive comeback. So how do centrosome aberrations in tumours occur, and how might they contribute to chromosomal instability (BOX 1) and other characteristic features of human tumours?

The centrosome duplication cycle

Discovered more than a century ago, the centrosome is a tiny organelle of surprising structural complexity (BOX 2). A single centrosome consists of two centrioles that are surrounded by amorphous pericentriolar material (PCM). Centrioles are important in the assembly of the PCM and the anchoring of microtubules, but the nucleation of microtubules occurs from within the PCM, where γ-TUBULIN RING COMPLEXES act as nucleation templates[14] (BOX 2). In humans and most other mammalian species, the sperm contributes the centrosome to the zygote[15]. Throughout development and adult life, this single centrosome then needs to be duplicated once, and only once, in every cell cycle. On the basis of early morphological studies, the centrosome duplication cycle can be subdivided into several distinct steps (BOX 3). Our understanding of the regulation of these steps remains

Summary

- The centrosome nucleates microtubules; it is important for cell shape, motility and division. During S phase of the cell cycle, the single centrosome that is present in a G1-phase cell is duplicated. The two centrosomes then set up the poles of the mitotic spindle and each incipient daughter cell receives one centrosome.
- The duplication and segregation cycles of centrosomes and chromosomes need to be coordinated to avoid chromosome missegregation or ploidy changes. The retinoblastoma pathway has been identified as one important link between centrosome duplication and chromosome replication.
- Many tumours display numerical and structural centrosome aberrations. Extra copies of centrosomes could, in principle, arise through overduplication within a single cell cycle, through aborted cell division, cell fusion or *de novo* genesis. A growing body of evidence points to aborted division as an important cause of excessive centrosome numbers.
- Cells that lack a functional p53 pathway are proposed to acquire multiple centrosomes through failure of a G1-phase checkpoint that should eliminate cells after aborted division. However, it has also been argued that p53 regulates centrosome duplication.
- Centrosome aberrations can give rise to chromosomal instability and altered tissue architecture. Importantly, centrosome aberrations and chromosomal instability are expected to enhance each other.
- Most multipolar divisions cause severe chromosome missegregation and therefore constitute lethal events. Occasionally, however, they might give rise to cells with chromosomal compositions that favour survival in the microenvironment of the tumour. In tumour cells, genes that are involved in alternative mechanisms for spindle formation might be upregulated or re-expressed. This might cause several centrosomes to coalesce and allow the formation of bipolar spindles, in spite of excessive centrosome numbers.
- A better understanding of the origins and consequences of centrosome aberrations could lead to the development of novel diagnostic, prognostic or therapeutic approaches.

incomplete, but it is clear that phosphorylation has a key role[4,5]. Furthermore, there is increasing evidence for an important contribution of ubiquitin-dependent proteolysis in the regulation of centrosome biology[16,17].

From the perspective of tumorigenesis, one of the key issues to be resolved concerns the coordination of the centrosome and chromosome duplication cycles (FIG. 1). Although these two cycles can be dissociated experimentally during the rapid early nuclear divisions in the embryos of some species[18], in human somatic cells they were shown to be linked through the retinoblastoma pathway[19]. Both centrosome duplication and DNA replication require the hyperphosphorylation of the retinoblastoma (RB) protein and the activation of cyclin-dependent kinase 2 (CDK2)[19–22]. This ensures one level of coordination between these two key S-phase events, but also implies that mutational inactivation of the retinoblastoma pathway in human cancers will potentially deregulate both DNA replication and centrosome duplication. Considering that the loss of coordination between the centrosome cycle and the chromosome cycle is likely to constitute an important primary cause of numerical chromosomal instability in human tumours, it is

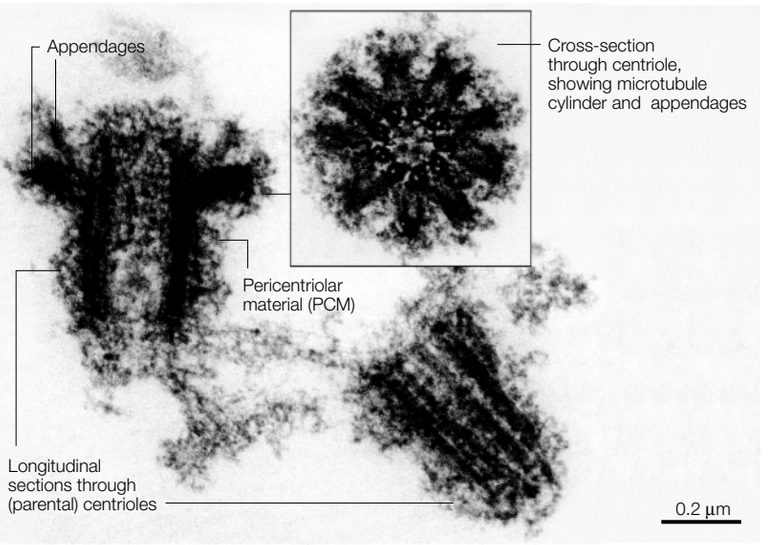
Box 1 | **Aneuploidy and chromosomal instability**

Most aggressive human cancers are characterized by an inherent instability of their genomes, a phenotype termed genomic (or genetic) instability[41,82–84]. Whether genomic instability is strictly required for tumorigenesis remains subject to debate[85,86], but it almost certainly favours both the adaptation of developing tumours to changing physiological conditions and the emergence of therapy-resistant cells. The most common type of instability — chromosomal instability — is visible at the cytological level. It is present in most, if not all, classes of solid tumour and so constitutes the most conspicuous hallmark of cancer[87]. As revealed by molecular cytogenetic methods, such as comparative genomic hybridization (CGH), multiplex fluorescence *in situ* hybridization (M-FISH) or spectral karyotyping (SKY), chromosomal instability is characterized by losses or gains of whole chromosomes (aneuploidy), as well as chromosome rearrangements. Importantly, the term 'chromosomal instability' describes a rate of change, whereas the term 'aneuploidy' merely refers to a state[83].

Relatively rare genomic instabilities occur at the level of the nucleotide sequence. These result from mutational inactivation of pathways that are involved in mismatch or nucleotide-excision repair[83]. By contrast, the molecular mechanisms that give rise to chromosomal instability remain largely unknown. Depending on the cause of instability, aberrant karyotypes might be dominated by either chromosome number aberrations or structural aberrations (such as amplifications, deletions and translocations). Many chromosome rearrangements might reflect telomere dysfunction, but chromosome number aberrations are likely to arise through different mechanisms. Centrosomal abnormalities almost certainly represent one important cause of chromosome missegregation[46,69,70]. Additional plausible causes include inappropriate chromosome condensation or cohesion, deregulated mitotic progression, an impairment of the spindle-assembly checkpoint, cytokinesis malfunction, endoreduplication or cell fusion[80,83,88,89].

γ-TUBULIN RING COMPLEX
A γ-tubulin-containing multiprotein complex that acts as a ring-shaped template for microtubule nucleation in metazoan organisms.

Box 2 | **Centrosome structure and function**



The centrosome is a relatively small organelle — its diameter is ~1 μm — that comprises a pair of centrioles that are embedded in a proteinaceous matrix of pericentriolar material (PCM) (see figure; adapted from REFS 90,91). Centrioles are cylindrical structures that are made up of nine triplet microtubules, but they are unequal in that only one (the older of the two) carries appendages that are close to its distal end (see figure). During S-phase of the cell cycle, procentrioles assemble next to the proximal ends of both parental centrioles (not shown). (Occasionally, parental centrioles and procentrioles are also referred to as mother and daughter centrioles, respectively.) The complete maturation of a centriole — that is, the time from procentriole formation to the acquisition of appendages — requires about 1.5 cell cycles. Centrioles are closely related to the basal bodies underlying cilia and flagella[1,3,92]. They contribute to PCM assembly and to the anchoring of microtubules, primarily via their appendages[1]. Centrioles are not strictly required for spindle formation, but are essential for the formation of spindle asters. In turn, interactions between astral microtubules and the cortex are crucial for spindle positioning, asymmetric cell divisions and morphogenesis during development[72].

Under the electron microscope, the PCM appears as an amorphous, electron-dense cloud (see figure). The complete inventory of centrosomal components has not yet been established, but several dozen proteins have been reported to localize to the centrosome, either transiently or throughout the cell cycle. Prominent among the PCM components are γ-tubulin ring complexes, which act as templates for microtubule nucleation[14]. In addition, the PCM harbours several large proteins with predicted coiled-coil domains (for example, AKAP450, kendrin/pericentrin, C-NAP1/CEP250, ninein and CEP135), indicating that these components perform structural functions[1–3]. Furthermore, several protein kinases, phosphatases, components of the ubiquitin-dependent proteolytic machinery and microtubule-dependent motors associate permanently or transiently with centrosomes[5,8]. Although many of these activities might control centrosome function, others might use the centrosome as a structural platform to enhance the efficiency of reactions that are crucial for cell-cycle progression. Remarkably, most centrosomal proteins that have been studied so far also exist in a soluble, cytoplasmic pool, indicating that centrosomes are highly dynamic structures.

Centrosomes organize the microtubule network throughout the cell cycle. During interphase, microtubule arrays direct the transport of membranous vesicle and organelles. Moreover, by interacting with the intermediate filament and actomyosin networks, they also influence cell shape, polarity and motility[93,94]. During mitosis, microtubules are indispensable for the formation of the spindle apparatus. In higher plants and specialized animal cells, notably female germ cells, spindles can form in the absence of centrosomes[75]. However, in most dividing animal cells, centrosomes instruct the formation of a bipolar spindle, and they determine the positioning of the contractile ring during cytokinesis. Furthermore, recent studies indicate that centrosomes are also required for abscission, the final stage of cell division[95], and for the subsequent G1 to S transition[6,76].

important to achieve a better understanding of the pathways that synchronize the two cycles in somatic cells.

Centrosome aberrations in human tumours

Centrosomal abnormalities are very common not only in tumour-derived cell lines and animal tumour models, but also in both primary and metastatic human tumours (FIG. 2). Extra copies of centrosomes (supernumerary centrosomes) have been described for nearly all cancers that have been surveyed, including brain, breast, bile duct, colon, head and neck, lung, pancreas and prostate cancers[11–13,23–29]. Increased centrosome numbers have also been reported for cervical cancers that are associated with high-risk human papillomavirus (HPV-16/HPV-18) infection[30–32]. This is a particularly interesting observation, as it offers a unique opportunity to explore the generation of centrosomal abnormalities in response to the expression of the HPV oncoproteins E6 and E7 (see below).

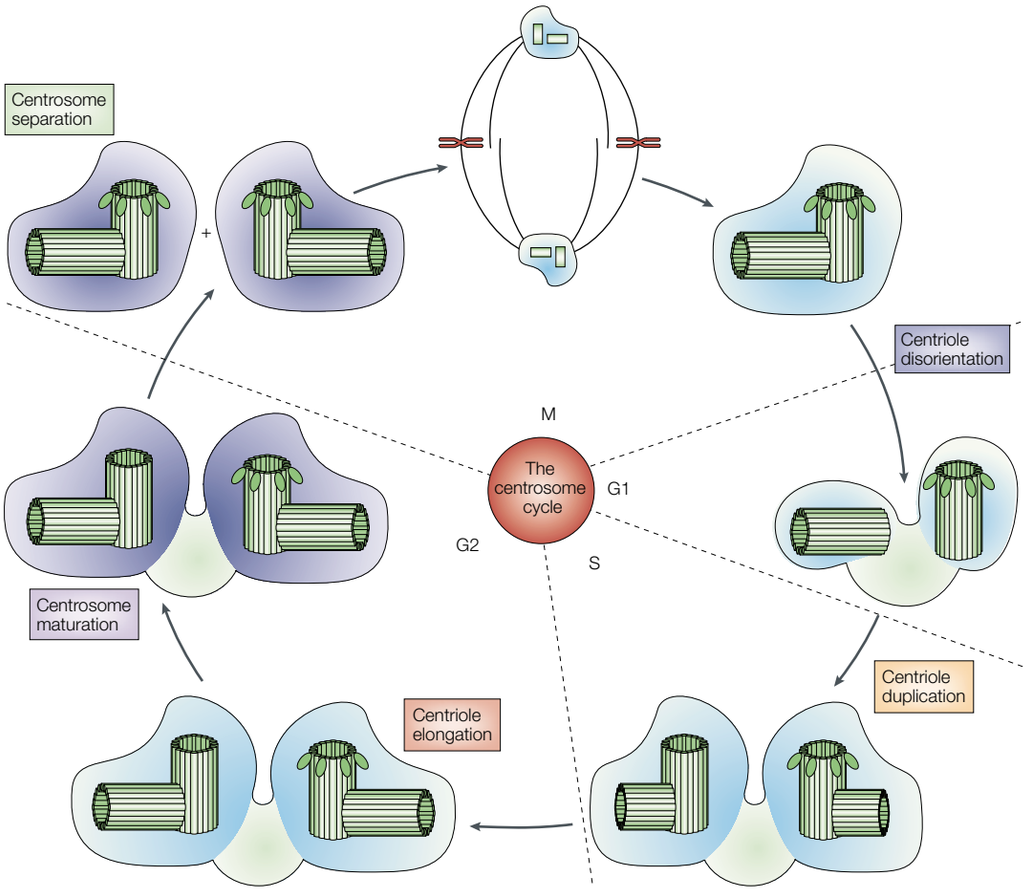
Numerical centrosome aberrations are frequently accompanied by structural irregularities. These include increases in centrosome size (FIG. 2), the formation of ACENTRIOLAR BODIES and alterations in the phosphorylation state of PCM components[11,12,18,23,26,31,33–35]. Presumably, these alterations reflect deregulation of the expression and activity of centrosomal proteins. Support for this

ACENTRIOLAR BODIES
Assemblies of centrosomal proteins that can form in the absence of centrioles.

idea comes from the demonstration that the overexpression of certain PCM components (such as pericentrin, TACC, CEP135 and C-NAP1) in cultured cells gives rise to structural centrosomal abnormalities that closely resemble those seen in tumours[23,36–39]. Furthermore, the overexpression of pericentrin in primary prostate epithelial cells reproduced several phenotypic characteristics of prostate tumours, notably increased genomic instability and loss of cellular architecture[23]. Adverse effects on spindle formation and chromosome segregation were also seen following overexpression of TACC and CEP135 (REFS 36,38), but not following overexpression of C-NAP1 (REF. 37). This illustrates that different types of PCM assemblies can exert diverse effects on centrosome function and cytoskeletal dynamics.

For most tumours, the functional consequences of structural centrosomal abnormalities remain to be explored. A recent survey that was performed on different types of breast cancer failed to reveal a correlation

Box 3 | **The centrosome cycle**



The centrosome duplication cycle can be subdivided into several discrete steps (see figure). During mitosis, the centrosome at each pole of the mitotic spindle contains a pair of centrioles. These two centrioles usually display a conspicuous orthogonal orientation, indicating that they are tightly connected. At the end of mitosis, this orthogonal association is lost during a process that is referred to as centriole disorientation. This step might relate to the final separation (abscission) of the two incipient daughter cells[95]. In addition, it might be required for the subsequent duplication step[21], or for the re-establishment of a linker structure between the two parental centrioles[96]. Centriole duplication then occurs during S phase. At the morphological level, this event is characterized by the formation of procentrioles at the proximal end of each parental centriole. So, duplication is semi-conservative from the perspective of the whole centrosome, but conservative from the perspective of the centriole[97]. How centriole duplication is brought about remains a mystery, but the recent establishment of *in vitro* assays for centrosome duplication might hopefully provide new opportunities for studying this fundamental problem[98,99]. Procentrioles then elongate until they reach their maximal length, but, importantly, the two centriole doublets continue to function as a single microtubule-organizing centre until late G2. At the G2–M transition, centrosome maturation occurs. This process involves the exchange of several PCM components and culminates in the recruitment of additional γ-tubulin ring complexes — a prerequisite for increased microtubule-nucleating activity. In response to the activation of microtubule-dependent motor proteins, centrosomes then separate from each other and instruct the formation of the two spindle poles. As a result, each incipient daughter cell again inherits one centrosome.

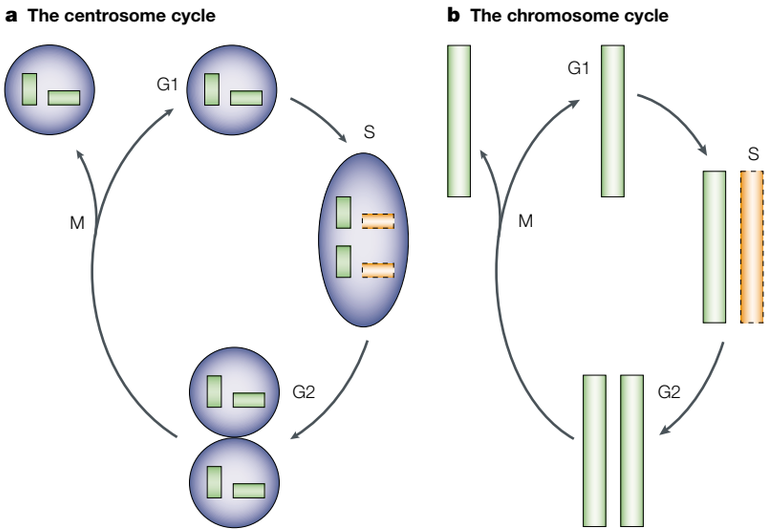


Figure 1 | **The cell cycle: a tale of two cycles**. A schematic comparison of **a** | the centrosome cycle and **b** | the chromosome cycle. Both the centrosome and the complete genome need to be duplicated once, and only once, in every cell cycle. Loss of coordination between the two cycles inevitably leads to chromosome missegregation or changes in ploidy.

between microtubule-nucleation capacity and centrosomal abnormalities[34]. This supports the view that the microtubule-nucleation ability of structurally aberrant centrosomes might be either reduced or enhanced, depending on the identity and modification state of the overexpressed PCM components. As different types of structural centrosomal abnormalities influence cellular properties in different ways, it might be rewarding to search for correlations between the overexpression of particular centrosomal proteins and clinical parameters that are associated with the corresponding tumours. Such correlations might constitute valuable prognostic indicators.

Chromosomal aberrations are particularly common in advanced, highly invasive cancers, and are increasingly used as a prognostic marker for tumour progression[40,41]. A similar situation might hold true for centrosomal abnormalities. As shown for lesions of the uterine cervix, breast and prostate, centrosomal abnormalities are common not only in highly advanced, invasive cancers, but can also be detected in low-grade tumours and *in situ* carcinomas[23,34,35]. Similarly, centrosome defects were found to represent an early event in the evolution of malignant phenotypes in ORGANOTYPIC CULTURE and animal models[42–45]. These studies support the hypothesis that centrosomal abnormalities constitute an important cause of chromosomal instability, rather than a secondary consequence of late-stage tumorigenesis. So, it is attractive to evaluate the utility of centrosomal markers as prognostic indicators for the development of aggressive forms of cancer[23,27,28].

Origins of centrosome aberrations?

Supernumerary centrosomes can arise through fundamentally distinct mechanisms[2,10,46]. As outlined in FIG. 3a, they might reflect several rounds of centrosome duplication within the same cell cycle (model I); however, as centrosome duplication requires several hours, this mechanism is expected to depend on a substantial delay in cell-cycle progression. Certain tumour-derived cell lines (for example, U2OS osteosarcoma cells) can indeed be induced to undergo several rounds of centrosome reduplication *in vitro*, provided that DNA replication is arrested for many hours by drugs such as hydroxyurea or aphidicolin[19,47]. Interestingly, however, other tumour-derived cell lines (for example, HeLa) arrest both centrosome duplication and DNA replication in response to the same drugs[48]. What determines this response is an important unresolved question[49]. In particular, it would be important to know whether cells normally possess a mechanism that limits centrosome duplication to once per cell cycle. If such a mechanism exists, one might expect it to be mutated in those tumour cell lines that re-duplicate centrosomes following inhibition of S-phase progression.

A second plausible scenario for the generation of cells with supernumerary centrosomes invokes an aborted cell division (FIG. 3a; model II). Cell-division failure can have several distinct primary causes, including

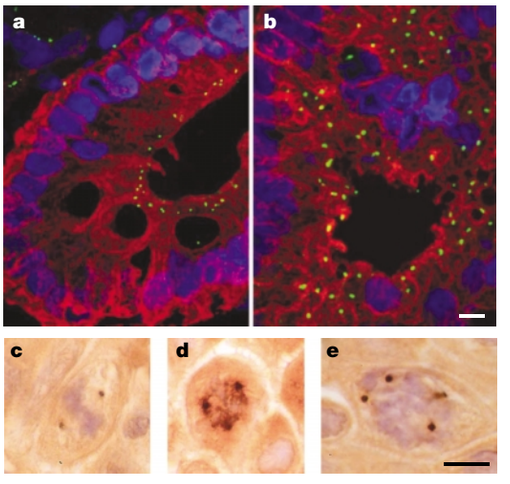


Figure 2 | **Centrosomal abnormalities in human tumours.** **a**,**b** | Normal (**a**) and tumour (**b**) colon tissues from the same patient were stained with antibodies against cytokeratin 20 (red) to identify epithelial cells, pericentrin (green) to label centrosomes, and with Hoechst 33342 (blue) to label nuclei. Normal crypt epithelial cells (**a**) have apical centrosomes and basal nuclei, with approximately one centrosome per nucleus. The aneuploid tumour (**b**) has amplified centrosomes that are larger and more numerous than those in the normal tissue. Moreover, cellular organization is disturbed. Bar denotes 10 μm. Images kindly provided by Vivian Negron and Wilma Lingle (Mayo Clinic, Rochester, Minnesota, USA). **c–e** | A human prostate tumour (**d**,**e**) and adjacent tissue (**c**) were sectioned, stained for γ-tubulin (brown), and processed for immunoperoxidase. Nuclei were stained with hematoxylin (purple). Compared with the bipolar spindle of a normal mitotic cell (**c**), the spindles in many dividing tumour cells are multipolar, with much larger γ-tubulin-positive poles (**d**,**e**). Bar denotes 10 μm. Images kindly provided by German Pihan and Stephen Doxsey (University of Massachusetts, Worcester, Massachusetts, USA).

ORGANOTYPIC CULTURE
The *in vitro* maintenance and growth of tissue explants and multicellular cultures that mimic cell interactions within tissues.

the persistence of unrepaired DNA damage or the deregulation of pathways that coordinate mitotic progression and cytokinesis. Another important reason for aborting division relates to the spindle-assembly checkpoint. This checkpoint delays the separation of sister chromatids (anaphase onset) until all chromosomes have undergone correct bipolar attachment on the spindle apparatus[50]. Malfunction of this checkpoint, or adaptation to a prolonged checkpoint arrest (mitotic slippage), will result in aberrant mitotic exit. Regardless of the primary cause of the failed division, the resulting G1 cell will contain not only twice the normal amount of DNA, but also twice the normal number of centrosomes. As discussed further below, the subsequent fate of such a cell — cell-cycle arrest, apoptosis or re-entry into S phase — seems to depend on the presence or absence of a functional p53 checkpoint pathway.

A third scenario for the generation of supernumerary centrosomes is based on cell fusion (FIG. 3a; model III). Although this mechanism has not yet received much attention in the context of tumorigenesis, fusion-induced centrosome amplification has been observed following ectopic expression of the RAD6 ubiquitin-conjugating enzyme in human breast epithelial cells[51]. Clearly, this mechanism could be important in cells that have been infected by viruses with fusogenic activities.

A fourth possible mechanism, not illustrated in FIG. 3, relates to the *de novo* formation of centrioles. The existence of pathways for *de novo* assembly of centrioles and basal bodies was long thought to be restricted to highly specialized cell types (such as multiciliated epithelial cells), but a recent study on the consequences of centrosome ablation by laser microsurgery indicates that many vertebrate somatic cells are also able to form centrioles *de novo* (REF. 109). This provocative finding indicates not only that mechanisms allowing the *de novo* formation of centrioles are more widespread than previously suspected, but also that these mechanisms are normally suppressed by existing centrioles. This implies that unscheduled activation of *de novo* assembly pathways could contribute to excessive centriole numbers in cancer cells.

The four described mechanisms for the generation of supernumerary centrosomes are not mutually exclusive, and the available evidence is not sufficient to definitively favour one mechanism over another. However, supernumerary centrosomes have been observed in response to deregulation (either knockout or overexpression) of several gene products that are implicated in human cancer (TABLE 1). It is striking that few, if any, of these genes are ostensibly involved in the regulation of the centrosome cycle. Although it is possible that future studies will reveal a link between these genes and centrosome duplication, at present it seems more straightforward to postulate that the deregulation of the genes listed in TABLE 1 could interfere with cell-cycle progression. A number of distinct primary defects, such as the persistence of unrepaired DNA damage, errors in chromosome structure or deregulated mitotic progression, could interfere with successful cell division. This would then lead to a similar terminal phenotype that is characterized by an increase in both chromosome and centrosome numbers (FIG. 3a; model II). So, aborted mitoses might constitute an important primary cause of numerical centrosome aberrations in tumours[48].

One way to further explore the relative importance of different mechanisms for generating extra copies of centrosomes in tumours is to examine the ploidy of the cells that show these abnormalities. Whereas a mechanism based on overduplication should initially produce

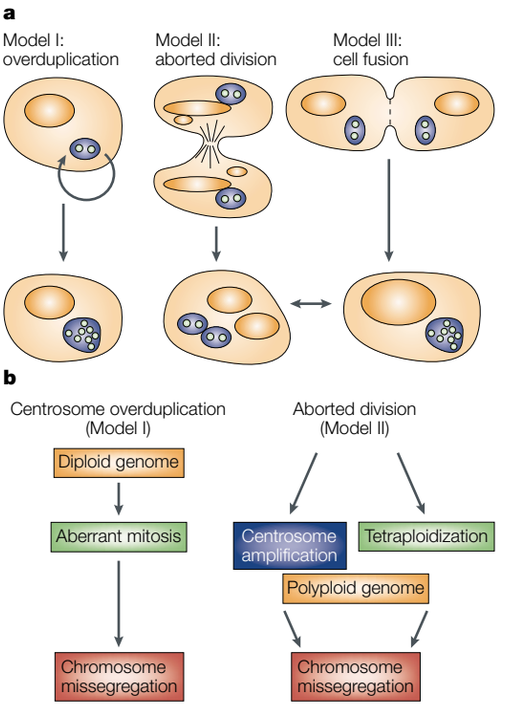


Figure 3 | **Centrosome amplification. a** | Mechanisms of centrosome amplification. Three plausible models for the generation of supernumerary centrosomes. A fourth model — *de novo* assembly of centrioles — is not indicated. For the sake of simplicity, all supernumerary centrosomes are shown in clusters, although scattered distributions might also be generated. Model I: deregulated centrosome duplication. Supernumerary centrosomes arise through several rounds of duplication within a single S phase. Model II: failure to complete cell division. As a result of an aborted mitosis, a tetraploid (or near-tetraploid) cell contains two centrosomes that are already in G1. Model III: cell fusion. Depending on the cell-cycle stages of the fusion partners, the products of such fusions will display different centrosome/genome ratios. Note that the products of fusion and aborted cell division will first be multinucleated, but often form single polyploid nuclei after subsequent mitoses. **b** | Centrosome amplification and ploidy. Centrosome overduplication during a prolonged S phase will give rise to supernumerary centrosomes in a diploid cell. In striking contrast, an aborted mitosis will generate supernumerary centrosomes that are concomitant with an increase in ploidy. Although supernumerary centrosomes are expected to cause chromosome missegregation in all dividing cells (regardless of ploidy), the likelihood of generating viable, potentially harmful progeny (in the form of hyperdiploid cells) is enhanced when segregating chromosomes of a tetraploid rather than a diploid genome. So, the combination of supernumerary centrosomes with tetraploidy sets the stage for chromosome missegration and chromosomal instability.

Table 1 | **Genes implicated in centrosome amplification***

Gene	Proposed function	References
p53 pathway		
p53 (knockout)	Cell-cycle checkpoint	55
WAF1 (antisense)	p53 target/CDK inhibitor	100
Gadd45 (knockout)	p53 target/checkpoint	101
Mdm2 (overexpression)	Ubiquitin-ligase for p53	29
DNA-repair pathway		
ATR (gene duplication)	Protein kinase/checkpoint	102
Brca1 (knockout)	DNA recombination	103
Brca2 (knockout)	DNA recombination	104
XRCC2/3 (mutation)	Recombination/repair	71
Protein degradation		
Tsg101 (knockout)	Ubiquitylation	105
Skp2 (knockout)	Ubiquitylation	106
RAD6 (overexpression)	Ubiquitylation/DNA repair	51
Mitosis		
Aurora-A (overexpression)	Protein kinase	107
Survivin (antisense)	Cytokinesis?	108

*Note that in some cases (for example, XRCC2/3), centrosome 'amplification' might primarily reflect fragmentation, rather than a true numerical aberration.

supernumerary centrosomes in (near-) diploid cells, numerical centrosome aberrations arising through aborted mitoses should be accompanied by an approximate doubling of chromosome content (FIG. 3b). Remarkably, a growing body of evidence indicates that tetraploidization frequently precedes aneuploidy in solid human tumours[52–54]. This is in line with a model in which aborted divisions give rise simultaneously to tetraploidy and supernumerary centrosomes (FIG. 3b).

Centrosome amplification and the p53 pathway. Much of the renewed interest in a possible link between centrosomal abnormalities and tumorigenesis was stimulated by the demonstration that the loss of the p53 tumour suppressor results in supernumerary centrosomes[55]. p53 is a transcription factor that causes cell-cycle arrest or apoptosis in response to DNA damage. A significant proportion (~10–30%) of $p53^{-/-}$ mouse embryo fibroblasts cultured *in vitro* have supernumerary centrosomes, and increased centrosome numbers have also been observed in mouse models that have impaired p53 pathways[13,29,56]. Furthermore, supernumerary centrosomes have been described following deletion of two p53 targets — the CDK2 inhibitor WAF1 (also known as p21) and GADD45 — and following overexpression of MDM2/HDM2, a ubiquitin-ligase and negative regulator of p53 (TABLE 1). So, it is well established that the loss of a functional p53 pathway favours the appearance of cells with supernumerary centrosomes, both in tissue culture and in tumours[29,55]. But what is the link between p53 function and the centrosome duplication cycle?

It has been argued that loss of p53 causes centrosome overduplication within a single S phase[55,57]. However, a recent study favours an alternative interpretation[48]. Overexpression of Aurora-A and other mitotic kinases was shown to cause centrosome amplification by interfering with the successful completion of cell division, giving rise to cells that were characterized by both centrosome amplification and polyploidy. Remarkably, both of these phenotypes were exacerbated in a $p53^{-/-}$ background[48]. Therefore, centrosome amplification in $p53^{-/-}$ cells does not necessarily imply a role for p53 in the regulation of centrosome duplication, but instead might reflect the involvement of a p53-dependent checkpoint in the elimination of cells that emerge from aborted divisions[48,58–63].

Several additional arguments support the view that the absence of p53 favours the emergence of supernumerary centrosomes through an indirect, checkpoint-related mechanism. Centrosome amplification is not an inevitable consequence of p53 deficiency *in vivo*[64], indicating that the elimination of p53 is not in itself sufficient to deregulate the centrosome cycle. Furthermore, the targeted inactivation of p53 in diploid human cells did not cause aneuploidy, although it favoured the formation of tetraploid cells[65]. It is also interesting to consider the generation of supernumerary centrosomes by the HPV-encoded oncoproteins, E6 and E7 (REF. 30). Whereas E7 primarily targets the retinoblastoma gene product (see below), E6 causes the ubiquitin-dependent degradation of p53. Yet, overexpression of E6 in primary human keratinocytes failed to exert a rapid effect on centrosome duplication, but instead produced centrosome amplification in conjunction with multinucleation[66,67]. Similarly, when expressed in a lung cancer cell line, E6 did not cause chromosomal instability unless mitotic-spindle formation was transiently abrogated[68].

Centrosome amplification and the RB pathway. Considering that both DNA replication and centrosome duplication are regulated through the RB pathway, it is attractive to speculate that mutational inactivation of this pathway — a common event in human tumours — could set the stage for centrosome overduplication[19]. However, although the loss of RB function might create permissive conditions for centrosome overduplication,

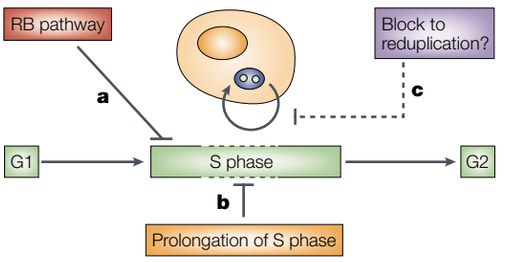


Figure 4 | **The RB pathway and centrosome duplication.** This speculative model proposes that overduplication of centrosomes within the same cell cycle depends on at least two (and possibly three) events. **a** | The mutational inactivation of the RB pathway might create a permissive environment for centrosome overduplication. **b** | A delay in S-phase progression could then provide the time that is required for several rounds of centriole duplication. This could result, for instance, from activation of an intra S-phase checkpoint (for example, in response to chemotherapy or γ-irradiation). **c** | If cells possess a pathway that normally prevents centrosome reduplication within the same cell cycle, this block to reduplication would also have to be relieved. Different human tumour cells might or might not possess such a pathway, and this could influence the outcome of therapeutic intervention.

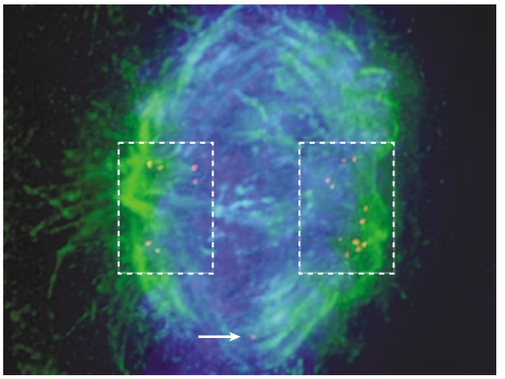


Figure 5 | **Coalescence of centrosomes to two poles in a mitotic neuroblastoma cell.** Centrosomes in a mitotic N115 neuroblastoma cell were stained with antibodies against centrin (orange). Microtubules were counter-stained with antibodies against α-tubulin (green) and DNA was labelled with Hoechst dye (blue). Note that most centrosomes have assembled to two broad spindle poles (boxed), but at least one centrosome (arrowhead) has not (yet) coalesced. Image kindly provided by Martina Casenghi (Max Planck Institute of Biochemistry, Martinsried, Germany).

this alone is clearly not sufficient (FIG. 4). Several rounds of centrosome duplication could only occur in RB-deficient cells if S phase was sufficiently prolonged, for instance, in response to activation of a DNA-damage checkpoint. Studies on the E7 oncoprotein of HPV seem consistent with a role of the RB pathway in restraining centrosome duplication[43,67].

So, the available evidence indicates that the E6 and E7 oncoproteins use distinct mechanisms for generating centrosome amplification and chromosomal instability[31]. By interfering with p53, E6 might favour the survival of cells exiting aberrant mitoses. E7 might inactivate RB and thereby set the stage for centrosome overduplication. If these interpretations are correct, then the two cooperating oncoproteins of high-risk HPV would trigger two major mechanisms for centrosome amplification.

Consequences of centrosome aberrations

Centrosome amplification and genetic instability. Because centrosomes have a dominant role in the formation of the mitotic-spindle apparatus, centrosomal abnormalities will almost inevitably cause the formation of abnormal spindles, with dire consequences for the integrity of the genome. Multipolar spindles are indeed common in tumours (FIG. 2). They often reflect excessive centrosome numbers[46,69,70], but acentriolar bodies can also occasionally act as spindle poles. Such bodies can arise through the assembly of overexpressed PCM components (as discussed above), or through centrosome fragmentation[71]. So, caution should be exerted when interpreting data that are based exclusively on the use of antibodies against components of the PCM; definitive evidence for centrosome amplification will generally require the visualization of centrioles. Monopolar spindles have also been described in tumours. These could theoretically arise as a result of failed centrosome duplication, but most frequently reflect defects in centrosome separation.

Analyses of human tumours have revealed a strong positive correlation between centrosomal abnormalities and chromosome number aberrations[12,24,26,30,32,34,45]. However, correlative evidence does not establish causality, and so far it has not been possible to directly show that centrosome abnormalities constitute a frequent primary cause of aneuploidy. Yet there is no doubt that the two phenotypes enhance each other; centrosome aberrations will foster chromosome missegregation, regardless of whether they arose through deregulation of the centrosome cycle or as a consequence of another primary event. Even those supernumerary centrosomes that result from aborted mitoses do not merely constitute innocent bystanders (and potentially useful markers) of tetraploidization. Instead, the presence of extra copies of centrosomes in tetraploid cells inevitably sets the stage for enhanced chromosome missegregation[48].

Centrosome aberrations and tissue architecture. So far, most studies on the link between centrosomes and tumours have focused on the impact of centrosomal abnormalities on the stability of the genome. But considering that loss of cell polarity and tissue architecture is an important aspect of tumour progression, other facets of centrosome function should not be neglected[23,34]. In particular, centrosome positioning determines both the orientation of the cleavage plane and the (a)symmetry of cell division, two parameters that are absolutely crucial in epithelia and stem-cell compartments[72–74]. Investigations into the impact of centrosomal abnormalities on tissue organization might therefore prove rewarding and will hopefully lead to a better understanding of the cytoskeletal changes that underlie the acquisition of an invasive phenotype.

Compensation for extra centrosomes?

Dysfunctional or supernumerary centrosomes will either impede cell division or cause multipolar divisions, which will most frequently lead to mitotic catastrophe. Neither of these phenotypes would be expected to favour the clonal expansion of a tumour cell. So why are centrosomal abnormalities so common in tumours? Why are they not eliminated by negative selection? Two observations might, together, provide an answer to these questions.

First, observation of established tumour cell lines *in vitro* reveals that cells with supernumerary centrosomes are usually present at comparatively low levels, amounting to just a small proportion (~1–15%) of the total cell population. These levels are fairly constant and almost certainly reflect a steady-state situation, which is determined by the rate at which cells with supernumerary centrosomes arise *de novo*, and the rate at which they die, due to either elimination by cell-cycle checkpoints or mitotic catastrophe. So, under *in vitro* culture conditions, the acquisition of extra copies of centrosomes generally constitutes a disadvantage. This situation is likely to be different *in vivo*, where the fraction of

cells with centrosome abnormalities progressively increases with advancing tumour stages[23,34,45]. Although most of the multipolar divisions that occur in tumours probably reflect non-productive events, an occasional division might give rise to progeny with a genetic constitution that favours survival in a changing physiological environment. Selective pressure might arise, for instance, through increasing hypoxia or nutritional deprivation in a growing tumour mass, or through the presence of a chemotherapeutic drug.

Second, tumour cells might divide successfully in spite of supernumerary centrosomes because of alternative, centrosome-independent mechanisms for bipolar spindle formation[75,76]. This point is illustrated best by neuroblastoma-derived cell lines that harbour large numbers of centrioles, and yet undergo mostly bipolar divisions, with often unequal numbers of centrioles coalescing at the two poles[77–79] (FIG. 5). Considering that not all supernumerary centrosomes (or acentriolar bodies) of tumour cells are necessarily equivalent[33], it is possible that only two pairs of centrioles are actually functional. Alternatively, however, it is attractive to speculate that certain tumours might compensate for the presence of supernumerary centrosomes by re-expressing or upregulating genes that code for important components of an alternative, centrosome-independent pathway for spindle formation. As a result, supernumerary centrosomes would be forced to coalesce into two spindle poles[46,69]. This would then allow the tumour to expand through binary divisions, while at the same time maintaining genetic instability through occasional multipolar divisions. If this hypothesis were correct, upregulated alternative mechanisms for bipolar spindle formation would constitute attractive drug targets.

Conclusions and future directions

The past several years have seen a surge of renewed interest in the hypothesis that centrosomal abnormalities might contribute to the development of cancer[10]. As yet, there is no genetic evidence to indicate that centrosomal abnormalities constitute a frequent cause of tumour initiation. However, centrosomal abnormalities are observed in early, pre-cancerous lesions, which supports the view that they fuel tumour progression. In principle, supernumerary centrosomes in tumours could arise through deregulation of the centrosome cycle. However, recent evidence indicates that they might frequently constitute a secondary consequence of cell-cycle deregulation. This by no means diminishes the importance of centrosome aberrations for tumorigenesis, as supernumerary centrosomes will foster chromosomal instability regardless of their origin. Whether this then implies that centrosome aberrations have a causal role in the development of cancer depends on the extent to which genomic instability is important for the outgrowth of malignant and drug-resistant cells[80]. Finally, it is important to emphasize that aberrant centrosomes could promote progression of cancers to more malignant forms not only through their impact on the stability of the genome, but also through their influence on tissue architecture. The exploration of this intriguing possibility has barely begun.

From a clinical perspective, centrosomal abnormalities are interesting for their potential use as diagnostic or prognostic markers[23]. Furthermore, it might prove rewarding to explore centrosome-related processes for their potential exploitation in therapeutic approaches. First, cells bearing aberrant centrosomes might offer therapeutic windows for drugs that are directed at centrosomes or microtubules[33]. Second, if alternative mechanisms for bipolar spindle formation were indeed upregulated in tumours with supernumerary centrosomes, such mechanisms would constitute attractive targets for therapeutic intervention. Third, if regulatory pathways normally prevent the reduplication of centrosomes within the same cell cycle, the inactivation of such pathways should favour centrosome overduplication following treatment of tumours with agents that extend the duration of S phase. Conceivably, this could increase the frequency of multipolar mitoses to a level that is no longer compatible with the survival of progeny[81].

So, where do we go from here? One important task for the future will be to explore the relative contribution of different mechanisms to the generation of supernumerary centrosomes in the course of tumour development. This will undoubtedly require the establishment of appropriate animal models. Another important goal is to elucidate the regulatory circuits that control the centrosome cycle. A detailed molecular understanding of the links that coordinate the duplication and segregation of centrosomes with the propagation of the genome will not only lead to a better appreciation of the role of centrosomes in human cancer, but might also provide a rational basis for the development of centrosome-related diagnostic or therapeutic applications.

1. Bornens, M. Centrosome composition and microtubule anchoring mechanisms. *Curr. Opin. Cell Biol.* **14**, 25–34 (2002).
2. Doxsey, S. Re-evaluating centrosome function. *Nature Rev. Mol. Cell Biol.* **2**, 688–698 (2001).
3. Lange, B. M. H. Integration of the centrosome in cell cycle control, stress response and signal transduction pathways. *Curr. Opin. Cell Biol.* **14**, 35–43 (2002).
4. Hinchcliffe, E. H. & Sluder, G. 'It takes two to tango': understanding how centrosome duplication is regulated throughout the cell cycle. *Genes Dev.* **15**, 1167–1181 (2001).
5. Meraldi, P. & Nigg, E. A. The centrosome cycle. *FEBS Lett.* **521**, 9–13 (2002).
6. Hinchcliffe, E. H., Miller, F. J., Cham, M., Khodjakov, A. & Sluder, G. Requirement of a centrosomal activity for cell cycle progression through G1 into S phase. *Science* **291**, 1547–1550 (2001).
Cell microsurgery is used to study the role of the centrosome during cell-cycle progression. This provocative study points to a crucial centrosome-related function at the G1–S transition.
7. Pines, J. Four-dimensional control of the cell cycle. *Nature Cell Biol.* **1**, E73–E79 (1999).
8. Rieder, C. L., Faruki, S. & Khodjakov,A. The centrosome in vertebrates: more than a microtubule-organizing center. *Trends Cell Biol.* **11**, 413–419 (2001).
9. Sibon, O. C., Kelkar, A., Lemstra, W. & Theurkauf, W. E. DNA-replication/DNA-damage-dependent centrosome inactivation in *Drosophila* embryos. *Nature Cell Biol.* **2**, 90–95 (2000).
10. Boveri, Th. *Zur Frage der Entstehung maligner Tumoren, 1914* (English Translation: The Origin of Malignant Tumors, Williams and Wilkins, Baltimore, Maryland, 1929).
A summary of the pioneering experiments that provide the foundations for our current thinking about the role of the centrosome in tumorigenesis. A lucid and prophetic treatise.
11. Lingle, W. L., Lutz, W. H., Ingle, J. N., Maihle, N. J. & Salisbury, J. L. Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. *Proc. Natl Acad. Sci. USA* **95**, 2950–2955 (1998).
12. Pihan, G. A. *et al.* Centrosome defects and genetic instability in malignant tumors. *Cancer Res.* **58**, 3974–3985 (1998).
13. Weber, R. G. *et al.* Centrosome amplification as a possible mechanism for numerical chromosome aberrations in

cerebral primitive neuroectodermal tumors with *TP53* mutations. *Cytogenet. Cell Genet.* **83**, 266–269 (1998).
14. Zheng, Y., Wong, M. L., Alberts, B. & Mitchison, T. Nucleation of microtubule assembly by a γ-tubulin-containing ring complex. *Nature* **378**, 578–583 (1995).
15. Simerly, C. *et al.* The paternal inheritance of the centrosome, the cell's microtubule-organizing center, in humans, and the implications for infertility. *Nature Med.* **1**, 47–52 (1995).
16. Freed, E. *et al.* Components of an SCF ubiquitin ligase localize to the centrosome and regulate the centrosome duplication cycle. *Genes Dev.* **13**, 2242–2257 (1999).
17. Wojcik, E. J., Glover, D. M. & Hays, T. S. The SCF ubiquitin ligase protein slimb regulates centrosome duplication in *Drosophila*. *Curr. Biol.* **10**, 1131–1134 (2000).
18. Sluder, G. & Hinchcliffe, E. H. The coordination of centrosome reproduction with nuclear events during the cell cycle. *Curr. Top. Dev. Biol.* **49**, 267–289 (2000).
19. Meraldi, P., Lukas, J., Fry, A. M., Bartek, J. & Nigg, E. A. Centrosome duplication in mammalian somatic cells requires E2F and Cdk2-cyclin A. *Nature Cell Biol.* **1**, 88–93 (1999).
20. Hinchcliffe, E. H., Li, C., Thompson, E. A., Maller, J. L. & Sluder, G. Requirement of Cdk2–cyclin E activity for repeated centrosome reproduction in *Xenopus* egg extracts. *Science* **283**, 851–854 (1999).
21. Lacey, K. R., Jackson, P. K. & Stearns, T. Cyclin-dependent kinase control of centrosome duplication. *Proc. Natl Acad. Sci. USA* **96**, 2817–2822 (1999).
22. Matsumoto, Y., Hayashi, K. & Nishida, E. Cyclin-dependent kinase 2 (Cdk2) is required for centrosome duplication in mammalian cells. *Curr. Biol.* **9**, 429–432 (1999).
23. Pihan, G. A. *et al.* Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. *Cancer Res.* **61**, 2212–2219 (2001).
A very careful study on the relationship between centrosome anomalies and prostate tumours at various stages of progression.
24. Lingle, W. L. & Salisbury, J. L. Altered centrosome structure is associated with abnormal mitoses in human breast tumors. *Am. J. Pathol.* **155**, 1941–1951 (1999).
Detailed ultrastructural analysis of centrosomes in human breast tumours, revealing a strong association between the excess of pericentriolar material and abnormal mitoses.
25. Sato, N. *et al.* Centrosome abnormalities in pancreatic ductal carcinoma. *Clin. Cancer Res.* **5**, 963–970 (1999).
26. Sato, N. *et al.* Correlation between centrosome abnormalities and chromosomal instability in human pancreatic cancer cells. *Cancer Genet. Cytogenet.* **126**, 13–19 (2001).
27. Kuo, K. K. *et al.* Centrosome abnormalities in human carcinomas of the gallbladder and intrahepatic and extrahepatic bile ducts. *Hepatology* **31**, 59–64 (2000).
28. Gustafson, L. M. *et al.* Centrosome hyperamplification in head and neck squamous cell carcinoma: a potential phenotypic marker of tumor aggressiveness. *Laryngoscope* **110**, 1798–1801 (2000).
29. Carroll, P. E. *et al.* Centrosome hyperamplification in human cancer: chromosome instability induced by p53 mutation and/or Mdm2 overexpression. *Oncogene* **18**, 1935–1944 (1999).
30. Duensing, S. & Munger, K. Centrosome abnormalities, genomic instability and carcinogenic progression. *Biochim. Biophys. Acta* **1471**, M81–M88 (2001).
31. Duensing, S. & Muenger, K. Human papillomaviruses and centrosome dupliation errors: modeling the origins of genomic instability. *Oncogene* **21**, 6241–6248 (2002).
32. Skyldberg, B. *et al.* Human papillomavirus infection, centrosome aberration, and genetic stability in cervical lesions. *Mod. Pathol.* **14**, 279–284 (2001).
33. Ghadimi, B. M. *et al.* Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations. *Genes Chromosom. Cancer* **27**, 183–190 (2000).
34. Lingle, W. L. *et al.* Centrosome amplification drives chromosomal instability in breast tumor development. *Proc. Natl Acad. Sci. USA* **99**, 1978–1983 (2002).
35. Pihan, G. A., Wallace, J., Zhou, Y. & Doxsey, S. Centrosome abnormalities and chromosome instability occur together in precancerous lesions. *Proc. Natl Acad. Sci. USA* (in the press).
36. Gergely, F. *et al.* The TACC domain identifies a family of centrosomal proteins that can interact with microtubules. *Proc. Natl Acad. Sci. USA* **97**, 14352–14357 (2000).
37. Mayor, T., Hacker, U., Stierhof, Y. D. & Nigg, E. A. The mechanism regulating the dissociation of the centrosomal protein C-Nap1 from mitotic spindle poles. *J. Cell Sci.* **115**, 3275–3248 (2002).
38. Ohta, T. *et al.* Characterization of Cep135, a novel coiled-coil centrosomal protein involved in microtubule organization in mammalian cells. *J. Cell Biol.* **156**, 87–100 (2002).
39. Purohit, A., Tynan, S. H., Vallee, R. & Doxsey, S. J. Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization. *J. Cell Biol.* **147**, 481–492 (1999).
40. Mitelman, F. Recurrent chromosome aberrations in cancer. *Mutat. Res.* **462**, 247–253 (2000).
41. Ried, T., Heselmeyer-Haddad, K., Blegen, H., Schrock, E. & Auer, G. Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. *Genes Chromosom. Cancer* **25**, 195–204 (1999).
42. Duensing, S. *et al.* Centrosome abnormalities and genomic instability by episomal expression of human papillomavirus type 16 in raft cultures of human keratinocytes. *J. Virol.* **75**, 7712–7716 (2001).
43. Duensing, S., Duensing, A., Crum, C. P. & Munger, K. Human papillomavirus type 16 E7 oncoprotein-induced abnormal centrosome synthesis is an early event in the evolving malignant phenotype. *Cancer Res.* **61**, 2356–2360 (2001).
44. Goepfert, T. M. *et al.* Centrosome amplification and overexpression of Aurora-A are early events in rat mammary carcinogenesis. *Cancer Res.* **62**, 4115–4122 (2002).
45. Shono, M. *et al.* Stepwise progression of centrosome defects associated with local tumor growth and metastatic process of human pancreatic carcinoma cells transplanted orthotopically into nude mice. *Lab. Invest.* **81**, 945–952 (2001).
46. Brinkley, B. R. Managing the centrosome numbers game: from chaos to stability in cancer cell division. *Trends Cell Biol.* **11**, 18–21 (2001).
47. Balczon, R. *et al.* Dissociation of centrosome replication events from cycles of DNA synthesis and mitotic division in hydroxyurea-arrested Chinese hamster ovary cells. *J. Cell Biol.* **130**, 105–115 (1995).
48. Meraldi, P., Honda, R. & Nigg, E. A. Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53$^{-/-}$ cells. *EMBO J.* **21**, 483–492 (2002).
Overexpression of Aurora-A and other mitotic kinases is shown to cause centrosome amplification, not by deregulating centrosome duplication as previously thought but, instead, through defects in cytokinesis that result in transiently tetraploid cells. This phenotype is enhanced in p53$^{-/-}$ cells.
49. Balczon, R. C. Overexpression of cyclin A in human HeLa cells induces detachment of kinetochores and spindle pole/centrosome overproduction. *Chromosoma* **110**, 381–392 (2001).
50. Millband, D. N., Campbell, L. & Hardwick, K. G. The awesome power of multiple model systems: interpreting the complex nature of spindle checkpoint signaling. *Trends Cell Biol.* **12**, 205–209 (2002).
51. Shekhar, M. P., Lyakhovich, A., Visscher, D. W., Heng, H. & Kondrat, N. Rad6 overexpression induces multinucleation, centrosome amplification, abnormal mitosis, aneuploidy, and transformation. *Cancer Res.* **62**, 2115–2124 (2002).
52. Galipeau, P. C. *et al.* 17p (p53) allelic losses, 4N (G2/tetraploid) populations, and progression to aneuploidy in Barrett's esophagus. *Proc. Natl Acad. Sci. USA* **93**, 7081–7084 (1996).
53. Shackney, S. E. *et al.* Model for the genetic evolution of human solid tumors. *Cancer Res.* **49**, 3344–3354 (1989).
54. Southern, S. A., Evans, M. F. & Herrington, C. S. Basal cell tetrasomy in low-grade cervical squamous intraepithelial lesions infected with high-risk human papillomaviruses. *Cancer Res.* **57**, 4210–4213 (1997).
55. Fukasawa, K., Choi, T., Kuriyama, R., Rulong, S. & Vande Woude, G. F. Abnormal centrosome amplification in the absence of p53. *Science* **271**, 1744–1747 (1996).
An influential study that contributed greatly to the revival of interest in the possible contribution of centrosome aberrations to carcinogenesis.
56. Levine, D. S., Sanchez, C. A., Rabinovitch, P. S. & Reid, B. J. Formation of the tetraploid intermediate is associated with the development of cells with more than four centrioles in the elastase-simian virus 40 tumor antigen transgenic mouse model of pancreatic cancer. *Proc. Natl Acad. Sci. USA* **88**, 6427–6431 (1991).
57. Tarapore, P., Horn, H. F., Tokuyama, Y. & Fukasawa, K. Direct regulation of the centrosome duplication cycle by the p53-p21Waf1/Cip1 pathway. *Oncogene* **20**, 3173–3184 (2001).
58. Andreassen, P. R., Lohez, O. D., Lacroix, F. B. & Margolis, R. L. Tetraploid state induces p53-dependent arrest of nontransformed mammalian cells in G1. *Mol. Biol. Cell* **12**, 1315–1328 (2001).
59. Borel, F., Lohez, O. D., Lacroix, F. B. & Margolis, R. L. Multiple centrosomes arise from tetraploidy checkpoint failure and mitotic centrosome clusters in p53 and RB pocket protein-compromised cells. *Proc. Natl Acad. Sci. USA*. **99**, 9819–9824 (2002).
60. Casenghi, M. *et al.* p53-independent apoptosis and p53-dependent block of DNA rereplication following mitotic spindle inhibition in human cells. *Exp. Cell Res.* **250**, 339–350 (1999).
61. Khan, S. H. & Wahl, G. M. p53 and pRb prevent rereplication in response to microtubule inhibitors by mediating a reversible G1 arrest. *Cancer Res.* **58**, 396–401 (1998).
62. Lanni, J. S. & Jacks, T. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. *Mol. Cell. Biol.* **18**, 1055–1064 (1998).
63. Minn, A. J., Boise, L. H. & Thompson, C. B. Expression of Bcl-x_L and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. *Genes Dev.* **10**, 2621–2631 (1996).
64. Goepfert, T. M. *et al.* Progesterone facilitates chromosome instability (aneuploidy) in p53 null normal mammary epithelial cells. *FASEB J.* **14**, 2221–2229 (2000).
65. Bunz, F. *et al.* Targeted inactivation of p53 in human cells does not result in aneuploidy. *Cancer Res.* **62**, 1129–1133 (2002).
66. Duensing, S. *et al.* The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. *Proc. Natl Acad. Sci. USA* **97**, 10002–10007 (2000).
67. Duensing, S., Duensing, A., Crum, C. P. & Munger, K. Human papillomavirus type 16 E7 oncoprotein-induced abnormal centrosome synthesis is an early event in the evolving malignant phenotype. *Cancer Res.* **61**, 2356–2360 (2001).
An interesting studying indicating that the two oncoproteins that are encoded by HPV-16 induce numerical centrosome aberrations by distinct mechanisms.
68. Haruki, N. *et al.* Persistent increase in chromosome instability in lung cancer: possible indirect involvement of p53 inactivation. *Am. J. Pathol.* **159**, 1345–1352 (2001).
69. Lingle, W. L. & Salisbury, J. L. The role of the centrosome in the development of malignant tumors. *Curr. Top. Dev. Biol.* **49**, 313–329 (2000).
70. Pihan, G. A. & Doxsey, S. J. The mitotic machinery as a source of genetic instability in cancer. *Semin. Cancer Biol.* **9**, 289–302 (1999).
71. Griffin, C. S., Simpson, P. J., Wilson, C. R. & Thacker, J. Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation. *Nature Cell Biol.* **2**, 757–761 (2000).
72. Knoblich, J. A. Asymmetric cell division during animal development. *Nature Rev. Mol. Cell Biol.* **2**, 11–20 (2001).
73. Meads, T. & Schroer, T. A. Polarity and nucleation of microtubules in polarized epithelial cells. *Cell Motil. Cytoskeleton* **32**, 273–288 (1995).
74. Reinsch, S. & Karsenti, E. Orientation of spindle axis and distribution of plasma membrane proteins during cell division in polarized MDCKII cells. *J. Cell Biol.* **126**, 1509–1526 (1994).
75. Karsenti, E. & Vernos, I. The mitotic spindle: a self-made machine. *Science* **294**, 543–547 (2001).
76. Khodjakov, A. & Rieder, C. L. Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. *J. Cell Biol.* **153**, 237–242 (2001).
This fascinating study examines — using laser microsurgery — the role of centrosomes during vertebrate cell-cycle progression. Following ablation of centrosomes, cells can still form bipolar mitotic spindles, but they frequently fail cytokinesis and they cannot reinitiate DNA synthesis.
77. Brinkley, B. R. *et al.* Tubulin assembly sites and the organization of cytoplasmic microtubules in cultured mammalian cells. *J. Cell Biol.* **90**, 554–562 (1981).
78. Ring, D., Hubble, R. & Kirschner, M. Mitosis in a cell with multiple centrioles. *J. Cell Biol.* **94**, 549–556 (1982).
79. Sharp, G. A., Weber, K. & Osborn, M. Centriole number and process formation in established neuroblastoma cells and primary dorsal root ganglion neurones. *Eur. J. Cell Biol.* **29**, 97–103 (1982).
80. Jallepalli, P. V & Lengauer, C. Chromosome segregation and cancer: cutting through the mystery. *Nature Rev. Cancer* **1**, 109–117 (2001).
81. Sato, N. *et al.* A possible role for centrosome overduplication in radiation-induced cell death. *Oncogene* **19**, 5281–5290 (2000).
82. Mantel, C. *et al.* p21(cip-1/waf-1) deficiency causes

deformed nuclear architecture, centriole overduplication, polyploidy, and relaxed microtubule damage checkpoints in human hematopoietic cells. *Blood* **93**, 1390–1398 (1999).
83. Hollander, M. C. *et al.* Genomic instability in Gadd45a-deficient mice. *Nature Genet.* **23**, 176–184 (1999).
84. Smith, L. *et al.* Duplication of ATR inhibits MyoD, induces aneuploidy and eliminates radiation-induced G1 arrest. *Nature Genet.* **19**, 39–46 (1998).
85. Xu, X. *et al.* Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Mol. Cell* **3**, 389–395 (1999).
86. Tutt, A. *et al.* Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification. *Curr. Biol.* **9**, 1107–1110 (1999).
87. Xie, W., Li, L. & Cohen, S. N. Cell cycle-dependent subcellular localization of the TSG101 protein and mitotic and nuclear abnormalities associated with TSG101 deficiency. *Proc. Natl Acad. Sci. USA* **95**, 1595–1600 (1998).
88. Nakayama, K. *et al.* Targeted disruption of Skp2 results in accumulation of cyclin E and p27(Kip1), polyploidy and centrosome overduplication. *EMBO J.* **19**, 2069–2081 (2000).
89. Zhou, H. *et al.* Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nature Genet.* **20**, 189–193 (1998).
90. Li, F. *et al.* Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. *Nature Cell Biol.* **1**, 461–466 (1999).
91. Gray, J. W. & Collins, C. Genome changes and gene expression in human solid tumors. *Carcinogenesis* **21**, 443–452 (2000).
92. Lengauer, C., Kinzler, K. W. & Vogelstein, B. Genetic instabilities in human cancers. *Nature* **396**, 643–649 (1998).
93. Loeb, L. A. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res.* **51**, 3075–3079 (1991).
94. Duesberg, P. & Rasnick, D. Aneuploidy, the somatic mutation that makes cancer a species of its own. *Cell Motil. Cytoskeleton* **47**, 81–107 (2000).
95. Tomlinson, I., Sasieni, P. & Bodmer, W. How many mutations in a cancer? *Am. J. Pathol.* **160**, 755–758 (2002).
96. Mitelman, F., Johansson, B. & Mertens, F. *Catalog of Chromosome Aberrations in Cancer* Vol. 2 (Wiley–Liss, New York, 1994).
97. Hartwell, L. H. & Kastan, M. B. Cell cycle control and cancer. *Science* **266**, 1821–1828 (1994).
98. Nigg, E. A. Mitotic kinases as regulators of cell division and its checkpoints. *Nature Rev. Mol. Cell Biol.* **2**, 21–32 (2001).
99. Bornens, M., Paintrand, M., Berges, J., Marty, M. C. & Karsenti, E. Structural and chemical characterization of isolated centrosomes. *Cell Motil. Cytoskeleton* **8**, 238–249 (1987).
100. Paintrand, M., Moudjou, M., Delacroix, H. & Bornens, M. Centrosome organization and centriole architecture: their sensitivity to divalent cations. *J. Struct. Biol.* **108**, 107–128 (1992).
101. Beisson, J. & Jerka-Dziadosz, M. Polarities of the centriolar structure: morphogenetic consequences. *Biol. Cell* **91**, 367–378 (1999).
102. Fuchs, E. & Cleveland, D. W. A structural scaffolding of intermediate filaments in health and disease. *Science* **279**, 514–519 (1998).
103. Schliwa, M., Euteneuer, U., Graf, R. & Ueda, M. Centrosomes, microtubules and cell migration. *Biochem. Soc. Symp.* **65**, 223–231 (1999).
104. Piel, M., Nordberg, J., Euteneuer, U. & Bornens, M. Centrosome-dependent exit of cytokinesis in animal cells. *Science* **291**, 1550–1553 (2001).
The careful examination of the behaviour of centrosomes in living mitotic cells revealed a remarkable repositioning of the older centriole to the midbody, indicating a crucial role for a centrosome-dependent pathway at the final stage of cell division.
105. Mayor, T., Stierhof, Y. D., Tanaka, K., Fry, A. M. & Nigg, E. A. The centrosomal protein C-Nap1 is required for cell cycle-regulated centrosome cohesion. *J. Cell Biol.* **151**, 837–846 (2000).
106. Kochanski, R. S. & Borisy, G. G. Mode of centriole duplication and distribution. *J. Cell Biol.* **110**, 1599–1605 (1990).
A very elegant study, demonstrating that tubulin incorporation into centrioles during each cell cycle is conservative, whereas the distribution of centrioles is semi-conservative.
107. Hinchcliffe, E. H. & Sluder, G. Centrosome reproduction in *Xenopus* lysates. *Methods Cell Biol.* **67**, 269–287 (2001).
108. Piel, M. & Bornens, M. Centrosome reproduction *in vitro*: mammalian centrosomes in *Xenopus* lysates. *Methods Cell Biol.* **67**, 289–304 (2001).
109. Khodjakov, A. *et al. De novo* formation of centrosomes in vertebrate cells arrested during S phase. *J. Cell Biol.* **158**, 1171–1181 (2002).

Acknowledgments
I thank F. Barr, T. Mayer, P. Meraldi, H. Silljé and C. Wilkinson for helpful comments on the manuscript and M. Bornens (Paris), M. Casenghi (Martinsried), S. Doxsey (Worcester) and W. Lingle (Rochester) for generously contributing material for the figures. My sincere apologies go to all authors whose primary work could not be cited due to space constraints.

Online links

DATABASES
The following terms in this article are linked online to:
Cancer.gov: http://www.cancer.gov/cancer_information/
bile duct cancer | brain cancer | breast cancer | cervical cancer | colon cancer | head and neck cancer | lung cancer | pancreatic cancer | prostate cancer
GenBank: http://www.ncbi.nih.gov/Genbank/
E6 | E7 | HPV-16
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
AKAP450 | Aurora-A | CDK2 | C-NAP1 | GADD45 | kendrin | MDM2 | ninein | p53 | pericentrin | RAD6 | RB | WAF1

FURTHER INFORMATION
Erich A. Nigg's web site:
http://www.biochem.mpg.de/nigg/home.shtml
Mitelman Database of Chromosome Aberrations in Cancer:
http://cgap.nci.nih.gov/Chromosomes/Mitelman
Mitosis World:
http://www.bio.unc.edu/faculty/salmon/lab/mitosis/mitosislabs.html
Access to this interactive links box is free online.