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Mitotic chromosomal instability and cancer: mouse modelling of the human disease

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Abstract

The stepwise progression from an early dysplastic lesion to full-blown metastatic malignancy is associated with increases in genomic instability. Mitotic chromosomal instability — the inability to faithfully segregate equal chromosome complements to two daughter cells during mitosis — is a widespread phenomenon in solid tumours that is thought to serve as the fuel for tumorigenic progression. How chromosome instability (CIN) arises in tumours and what consequences it has are still, however, hotly debated issues. Here we review the recent literature with an emphasis on models that recapitulate observations from human disease.

Since Boveri observed abnormal chromosome complements in tumour cells at the beginning of the twentieth century[1,2], the role of chromosome instability (CIN) in tumour initiation and progression has been a central issue in cancer biology. Only recently, using sophisticated mouse modelling approaches, is it becoming clear that CIN is not simply a passenger phenotype but probably plays a causative part in a substantial proportion of malignancies. However, several questions and controversies still remain. Here we review these issues through the analysis of recent findings and their relevance to human disease. We focus on two crucial questions: first, how is aneuploidy generated? Second, what is the role of CIN in tumour initiation and/or progression?

Throughout this Review we will concentrate on the CIN that arises as a result of an abnormal mitosis. This CIN can occur because of alterations in mitotic timing, mitotic checkpoint control, or of microtubule or centrosome dynamics. Abnormalities in double-

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/gene

Apc | ATM | Cdkn2a^{ARF} | CENPA | Cenpe | FBXO5 | Fzr1 | KIFC1 | MLH1 | MSH2 | NDC80 | Plk4 | Rae1 | RB1 | Rbl1 | Rbl2

OMIM: http://www.ncbi.nlm.nih.gov/omim

ataxia–telangiectasia | Li–Fraumeni | mosaic variegated aneuploidy

UniProtKB: http://www.uniprot.org

AURKA | BUB1 | BUB3 | BUBR1 | CDC20 | CDK1 | cyclin A | cyclin B1 | KIF11 | MAD1 | MAD2 | MPS1 | NEK2 | p53 | RANBP2 | SCC1 | securin | separase | UBCH10

FURTHER INFORMATION

Robert Benezra's homepage: http://www.mskcc.org/mskcc/html/10469.cfm

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strand break repair or telomere maintenance can also eventually lead to CIN as a result of repeated chromosome breakage–fusion–bridge cycles. Because of space constraints, however, we will not cover the deregulation of these pathways, although we note that they may ultimately lead to mitotic abnormalities. Failure of the mitotic checkpoint machinery, which blocks the separation of sister chromatids before microtubule attachment (described below), has been an obvious candidate mechanism involved in the generation of CIN during mitosis. Disappointingly, the mitotic checkpoint is rarely found to be compromised in human tumours. It is, however, frequently hyperactivated in chromosomally unstable lesions and, importantly, this overactivation of the mitotic checkpoint is intricately linked to the inhibition of major tumour suppressive pathways and the acquisition of CIN.

As a point of nomenclature, distinctions have previously been made between whole chromosome instability (W-CIN) and CIN that includes translocations, interstitial deletions and amplifications (segmental chromosome instability or S-CIN)[3]. S-CIN is observed in several model systems into which mitotic defects have been introduced[4–6]. Some models of CIN only show W-CIN changes, yet human tumours often contain both abnormal chromosome complements and structural changes, and so we prefer to use the global definition of CIN in this Review. Where indicated, we will distinguish between the two forms as W-CIN and S-CIN. Other defects in genome integrity, such as microsatellite instability, defects in nucleotide excision repair or base excision repair, defects in telomere maintenance or the stability of larger repeats and abnormalities in the G2/M DNA damage checkpoint, also play a part in tumour initiation and progression and have been reviewed extensively elsewhere[7–11].

Many of the studies that model mitotic CIN in mice are based on perturbations in the mitotic checkpoint pathway, which ensures the accurate segregation of chromosomes during mitosis. Because of its relevance to understanding genomic stability, a brief overview of the history of the identification of checkpoints is included in BOX 1. We reiterate, however, that although the identification of a DNA damage checkpoint and a mitotic checkpoint in yeast primed the cancer field to search for checkpoint mutations in human tumours, the requirement for the mitotic checkpoint in every cell division and the lethality observed in its absence make its loss in tumours an unlikely mechanism for the generation of CIN.

Box 1

A brief history of the identification of checkpoints

In the late 1980s Ted Weinert and Leland Hartwell identified the existence of a DNA damage checkpoint in budding yeast (*Saccharomyces cerevisiae*)[98]. The Rad9 mutant strain they characterized was shown to be defective in the ability to arrest cell division as a result of irradiation-induced DNA damage. Importantly, Rad9 mutants were viable in the absence of DNA damage; only when irradiated with ionizing radiation did the lethality become evident. Moreover, ionizing radiation-induced lethality could be rescued by providing sufficient time for repair by growing the cells in the presence of the microtubule poison benzimidazole, which we now know leads to activation of the mitotic checkpoint. These studies provided experimental evidence for the existence of cell cycle checkpoints[99]. These signalling pathways are postulated to be non-essential in the

absence of damage, and their main function is to arrest cell division until the damage is repaired or, in the case of mammalian cells, undergo programmed cell death when repair is not completed. Soon after these findings, two laboratories simultaneously identified an equivalent checkpoint responsible for arresting cell division in *S. cerevisiae* in response to mitotic spindle abnormalities induced by microtubule poisons. The mitotic arrest deficient (Mad)[100] and budding uninhibited by benzimidazole (Bub)[101] genes were identified in screens for sensitivity to spindle poisons and, as such, loss-of-function mutants were viable so long as cell division proceeded normally. These studies provided direct evidence for the existence of a spindle assembly checkpoint or mitotic checkpoint in budding yeast, the function of which is to arrest cell division at metaphase until all kinetochores are attached to microtubules from opposite spindle poles.

The later characterization of the mitotic checkpoint in mammalian cells[102] revealed important differences relative to budding yeast. First, the mitotic checkpoint is essential in all normal or transformed mammalian cells examined[49,53,103,104]. This unexpected result may be due to karyotypic complexity and the transient requirement of the mitotic checkpoint during an unperturbed cell cycle to prevent an intolerable level of chromosome instability. In addition, non-kinetochore-bound human MAD2 and BUBR1 have checkpoint-independent functions that are required to prevent premature exit from mitosis, perhaps by blocking the degradation of key substrates early in the mitotic cycle[23,28,105]. Therefore, unlike the case in budding yeast, mitotic checkpoint genes are generally essential in each mammalian cell division and, in contrast to the DNA damage checkpoint, their complete loss is unlikely to account for the accumulation of genomic damage in human tumours.

An outline of the mitotic checkpoint

The molecular mechanisms responsible for the mitotic checkpoint have been thoroughly reviewed elsewhere[12,13]. Here, we present a broad outline (FIG. 1), emphasizing some of the recent controversies.

In its simplest form, the mitotic checkpoint is a mechanism by which eukaryotic cells arrest cell division at metaphase until all sister kinetochores are attached to microtubules from opposite spindle poles. In prometaphase, sister chromatids are topologically linked by the ring-like cohesin complexes[14]. In addition, the activity of cyclin-dependent kinase 1 (CDK1, also known as cell division cycle 2; CDC2), the main mitotic kinase, is high and maintains the mitotic state. As mammalian cells proceed from prometaphase to metaphase, a signalling complex that contains mitotic arrest deficient 1 (MAD1), MAD2, MPS1 (also known as TTK), BUB1, BUB3 and BUBR1 assembles at unoccupied kinetochores. This in turn leads to the generation of a diffusible signal that is dependent on MAD2 and BUBR1 (REFS 15–19), which prevents the E3 ubiquitin ligase complex anaphase promoting complex/cyclosome (APC/C)[20] from degrading its mitotic targets cyclin B1 and securin (also known as pituitary tumour-transforming 1; PTTG1). In this state, exit from mitosis and the separation of sister chromatids are inhibited. As soon as the last kinetochore pair is attached to the microtubules at opposite spindle poles, the inhibitory diffusible signal is extinguished

and the APC/C is fully activated through the release of inhibition of its cofactor, CDC20. This leads to the ubiquitylation of cyclin B1 and securin, the two crucial partners of CDK1 and the cysteine protease separase (also known as ESPL1), respectively. Degradation of cyclin B1 by the 26S proteasome leads to a rapid decline in CDK1 activity, allowing exit from mitosis. Securin is a small inhibitory chaperone of separase, the activity of which is essential for the dissolution of cohesin complexes at and near sister chromatid kinetochores. Degradation of securin by the 26S proteasome and release from inhibition by CDK1–cyclin B1 phosphorylation owing to cyclin B1 degradation leads conjunctly to activation of separase and cleavage of the SCC1 (also known as RAD21) component of cohesin; the net effect of this is the separation of sister chromatids. Both of these events, inhibition of CDK1 and activation of separase, are necessary for a correct metaphase-to-anaphase transition and faithful segregation of chromosomes.

Although it is clear that a diffusible inhibitory signal is generated at kinetochores and prevents APC/C acting on cyclin B1 and securin[15], the nature of this event remains unclear. Musacchio and others have proposed a prion-like model based on two structural conformations adopted by MAD2 (REFS 21,22): open and closed. At this point the evidence for this model is biochemical but it accounts for the role of MAD2 at kinetochores, its interaction with CDC20 and the signal amplification required for the inhibition of the cell cycle by a single unoccupied kinetochore. Unoccupied kinetochores are known to recruit MAD1, which in turn binds with high affinity to MAD2 in its closed conformation. This MAD1–MAD2 complex is then thought to catalyse the conversion of open MAD2 monomers (the predominant form in the cytosol) to closed MAD2 forms that then bind to CDC20. This interaction serves a dual purpose: it inhibits the activity of APC/C (at least with regard to cyclin B1 and securin) and catalyses the further conversion of MAD2 open monomers to closed MAD2–CDC20 complexes, accounting for the required signal amplification.

Several observations substantially complicate this model. First, it is unclear what the role of BUBR1 is with regard to APC/C inhibition. BUBR1 is necessary for mitotic checkpoint function and is recruited to unoccupied kinetochores[23]. Its kinase domain is, however, dispensable for APC/C inhibition, and its amino (N)-terminal domain is sufficient to act as a pseudo-substrate inhibitor of the APC/C. Moreover, this APC/C inhibitory function is not dependent on the presence of BUBR1 at kinetochores. These findings have led to a modified MAD2 template model in which the heterodimeric CDC20–MAD2 closed conformer is required to deliver BUBR1 and perhaps BUB3 to the APC/C, where BUBR1 can then inhibit CDC20 function. The proposed mitotic checkpoint complex (MCC) composed of MAD2, CDC20, BUBR1 and BUB3 may be transient. MAD2 may depart, leaving behind a CDC20–BUBR1–BUB3 complex bound to the APC/C, although the duration of MAD2 persistence is still not completely resolved (for example, REFS 19,24).

Structural studies[25] suggest that CDC20 is displaced from its active location on the APC/C when MCC components are present; an event that may in turn facilitate CDC20 ubiquitylation[26], thereby maintaining a ‘checkpoint-on’ state. Here, ubiquitylation of CDC20 would continue so long as BUBR1–BUB3 (and perhaps MAD2) are still bound to the APC/C and would cease once the checkpoint is satisfied. Phosphorylation of MAD2

(REF. 27) and, recently, acetylation of BUBR1 (REF. 28) have been proposed to extinguish the binding to the APC/C and/or the stability of these components. This in turn would allow CDC20 to reoccupy its site on the APC/C, where it is protected from ubiquitylation. CDC20 would then assume its role in directing the APC/C to its principal downstream targets, securin and cyclin B1. Remarkably, cyclin A and NEK2 are ubiquitylated by the APC/C in the checkpoint-on state, adding further complexity to the inhibition of the APC/C. Substrate specificity and therefore substrate ordering seem to be key events in the different stages of mitosis but a molecular understanding of how substrate specificity arises remains limited.

In contrast to these findings, two independent reports have proposed that ubiquitylation of CDC20 by the E2 enzyme UBCH10 inactivates the checkpoint by blocking the association of CDC20 with MCC components[29,30]. The strongest evidence against this last model and in favour of one in which CDC20 ubiquitylation leads to its degradation during the checkpoint-on state comes from the finding that a form of CDC20 that lacks lysine and therefore cannot be ubiquitylated shows premature escape from mitotic arrest[26].

Reconciling all of these findings and integrating them into the MAD2 template model will certainly require more complex biochemical models but, more importantly, these models will need to be tested *in vivo* to understand how a single unoccupied kinetochore can maintain a cell, at least for a certain period of time (as we discuss below), in mitosis.

Aneuploidy and CIN in tumours

The notion that CIN contributes to tumour initiation and/or progression is as old as our understanding of chromosomes. As mentioned, Boveri postulated more than 100 years ago that abnormalities in chromosome segregation could promote tumour formation[1,2,26]. Although some arguments can still be made for aneuploidy as simply a passenger event in tumours, three lines of observation argue otherwise.

First, *in vitro* transformation of cell lines through various genetic alterations that lead to CIN suggests aneuploidy has a direct causal role in tumorigenesis. Transformation of cells in culture has been a standard assay to determine the oncogenic or tumour suppressive nature of a gene for more than two decades[31,32]. Although the genetic events that must occur for a primary cell to become transformed may differ substantially from those that occur in human tumours, several now-established oncogenes and tumour suppressors were identified by transformation assays[33–36]. Among the mitotic checkpoint genes with roles that have been explored in *in vitro* transformation, securin overexpression in primary cells leads to marked aneuploidy and is sufficient for transformation[37]. Overexpression of aurora kinase A (AURKA), the function of which is required for centrosome maturation, bipolar spindle assembly and mitotic entry[38], similarly leads to aneuploidy and transformation in human and rodent cells[39,40] as a result of abnormal mitoses.

Second, perhaps the most robust causative data linking CIN to tumorigenesis comes from the study of mouse models of aneuploidy. Several laboratories have generated mouse models of aneuploidy based on mutations or transcriptional changes of mitotic checkpoint genes observed in tumours. An obvious caveat of all these individual studies is that these genes

have non-mitotic functions that might explain their tumorigenic potential. In addition to their accepted mitotic functions, MAD2 (REF. 41) and RANBP2, a RAN GTPase binding protein that localizes to kinetochores during mitosis[42], have been implicated in nuclear trafficking. Securin has also been linked to modification of p53 function[43], and BUB1 and BUBR1 have been shown to play a part in the response to DNA damage[44,45]. Nevertheless, data from studies that have analysed various different genes involved in mitotic checkpoint control argue strongly for a contributory role of aneuploidy itself in tumour initiation and progression. TABLE 1 summarizes some of the mouse models of aneuploidy and their contributions to our understanding of CIN in human tumours. As we will describe in the next section, some of these models are more faithful to the mechanisms that are associated with CIN in human tumours than others but the message is the same: in general, CIN favours tumour formation.

Third, a large amount of data collected from human tumours suggests that aneuploidy has a causative role in tumorigenesis by showing that CIN and chromosomal aberrations correlate with tumour grade and prognosis[46,47]. Further supporting this argument, genes involved in maintaining chromosome stability are frequently deregulated in human tumours[48], as we will discuss in the next section. Finally, transcriptional expression profiles of aneuploid tumours have revealed a CIN signature that can be used to stratify lesions according to prognosis in an unbiased manner[46]. The fact that this CIN signature, which contains genes that are involved in a wide range of pathways, can predict clinical outcome even if genes that are regulated by the cell cycle are omitted provides further evidence that CIN plays a contributory part in the progression of these human tumours.

How is aneuploidy generated in human tumours?

Inevitably, most of the mechanistic studies that aim to answer this question have been carried out in mice and their results are summarized in TABLE 1. Many of these models have been generated based on the hypothesis that loss or downregulation of the mitotic checkpoint is responsible for CIN. Although this is largely the case *in vitro* and in model systems *in vivo*, as we discuss below, if these perturbations are to explain the mechanisms by which aneuploidy is generated in human cancer, there must be evidence for such changes in aneuploid human tumours. In other words, sufficiency for a cancer phenotype in mice or any other model cannot by itself be interpreted as an explanation for human disease without direct experimental evidence.

In mammalian cells a weakened mitotic checkpoint would be predicted to facilitate W-CIN as a result of premature exit from mitosis and premature separation of sister chromatids (FIG. 2). An overview of mouse models of aneuploidy reveals that this prediction is correct. Fibroblasts or lymphocytes derived from mice heterozygous for *Mad2l1* (which encodes MAD2)[49], *Bub1b* (which encodes BUBR1)[50,51], *Bub3* (REFS 52,53), *Bub1* (REF. 54) and centromere protein E (*Cenpe*)[55] or a *Cdc20*^{AAA} mutant that cannot bind MAD2 (REF. 56) show varied levels of aneuploidy. In addition, several animal strains that have these genetic lesions develop tumours in various organs at late stages of life or are more prone to tumours in sensitized backgrounds. Nevertheless, several separate lines of evidence argue against the

loss of mitotic checkpoint gene function as the main causative mechanism for aneuploidy in human tumours.

Through extensive analyses of aneuploid human tumours, it is now increasingly clear that mutations in mitotic checkpoint genes are rare (TABLE 1 and reviewed in REF. 57). As we have mentioned, complete loss of the mitotic checkpoint is lethal at the cellular and organismal levels. Note that conditional inactivation of *Bub1* in adult male mice[58] impairs fertility without any decrease in viability but no other tissues were examined, and it is unclear what the penetrance of inactivation was in this case. It therefore remains possible that actively proliferating tissues were primarily affected in this model and that the conditional *Bub1* mice survived because of incomplete penetrance. Downregulation of mitotic checkpoint genes, which is another putative mechanism for weakening the mitotic checkpoint, is also extremely rare. Importantly, the observations of decreased levels of mitotic checkpoint genes in cancer cell lines are often confounded by the lack of adequate controls, such as comparing the levels of MAD2 in various cancer cell lines with those of HeLa cells[59–62]. The expression of several genes that are required for mitosis and the mitotic checkpoint (*MAD2L1*, *BUB3*, polo-like kinases, *CDC20*, F-box protein 5 (*FBXO5,* also known as *EMI1*), *NDC80* (also known as *HEC1*), *PTTG1*, cyclin B1 (*CCNB1*), *CENPE* and *CENPA*, among others[63]) is under control of the E2f family of transcription factors and therefore can vary depending on the level of inhibition of the Rb pathway, the number of cells in G2/M phase of the cell cycle in an asynchronous population and the number of quiescent cells. These aspects all vary markedly between cell lines. How one defines a normal level of expression is also a key point here. It is reasonable to assume that the only adequate normal value is that of normal adjacent tissue to the primary tumour, provided the adjacent tissue is proliferating (which is seldom the case). In the case of cancer cell lines, normal adjacent tissue cannot be procured. Further confounding this issue, the levels of a mitotic checkpoint protein in a non-primary cell line relative to HeLa cells say little about the mitotic checkpoint status of that cell line. Indeed, there are few well-documented examples of robust functional mitotic checkpoint defects in tumour cells that have reduced expression of checkpoint proteins.

Several heritable cancer predisposition syndromes result from loss-of-function mutations in genes essential for the DNA damage checkpoint and DNA repair pathways. Li–Fraumeni syndrome (*TP53*), hereditary non-polyposis colorectal cancer (*MLH1* and *MSH2*), xeroderma pigmentosum (Xp family) and ataxia–telangiectasia (ataxia–telangiectasia mutated; *ATM*) are a few of the well-recognized ones. The existence of a range of these syndromes underscores two important points: first, that mutation of genes that control the DNA damage checkpoint and DNA repair pathways can be viable at a cellular, and often at an organismal level, and second, the accumulating DNA damage contributes to tumorigenesis. In the case of the mitotic checkpoint, only one genetic disorder has been associated with a mitotic checkpoint gene. Mosaic variegated aneuploidy (MVA) is an autosomal recessive disorder characterized by growth retardation, microcephaly and mosaic aneuploidies, predominantly monosomies and trisomies. Patients also show a high incidence of childhood tumours (Wilms' tumour, rhabdomyosarcoma and leukaemia). The disease has been genetically mapped to *BUBR1* (REF. 64), and CIN is thought to be the driving force for developmental defects and tumour formation. The severity of the phenotype in patients

with MVA and the lack of other related syndromes reinforce the notion that the mitotic checkpoint is crucial for normal organism growth and not just for the prevention of genomic abnormalities that result from external stress.

Finally, several cancer cell lines with marked CIN have a robust mitotic checkpoint when treated with microtubule-stabilizing drugs[65,66]. The strongest evidence against loss or downregulation of the mitotic checkpoint as a cause of aneuploidy in tumours is evident by looking at the transcriptional profiles of aneuploid tumours. In most cases, genes essential for the mitotic checkpoint are upregulated, sometimes to very high levels (Oncomine[48,67] and REF. 46). Although this may be the result of unrestricted proliferation in the absence of a functional Rb pathway, the consequence is not an absent or weakened checkpoint but, most likely, an overactive one[66]. Moreover, in retinoblastoma tissue samples, high levels of MAD2 are not confined to mitotic cells but are also found in interphase cells[68], arguing that it is not only the high mitotic index that contributes to MAD2 overexpression after Rb pathway inhibition.

CIN has long been known to be a dominant phenotype in cancer cell lines[69] and overactivation of the mitotic checkpoint in cancer cell lines fits this observation readily. It has also been proposed that aneuploidy might be an early event in cancer evolution, which induces a quasi-stable karyotypic state that is balanced by selection towards tumorigenesis[70]. Inhibition of the Rb pathway and the consequent overexpression of key mitotic checkpoint genes may efficiently initiate tumours because of this coupling of a loss of a tumour suppressor pathway to karyotypic instability.

Overexpression of MAD2 and HEC1 in inducible mouse models has already been shown to be sufficient for generating aneuploidy and initiating tumour formation[6,71]. In these models, hyperactivation of the mitotic checkpoint is predicted to lead to prolonged mitosis and the failure of one or more sister chromatids to separate on schedule. This would then increase the likelihood of merotelic attachments and lagging whole chromosomes or, in the extreme case, tetraploidy following mitotic slippage (also known as adaptation; FIG. 2). These events are readily seen in cells that overexpress *MAD2L1* or *NDC80*. Although overexpression of these mitotic genes might have nonmitotic consequences or off-target effects, a recent elegant study[72] has shown that prolonged activation of the mitotic checkpoint using spindle-stabilizing agents or the mitotic kinesin family member 11 (KIF11, also known as EG5) inhibitor monastrol also leads to lagging sister chromatids, merotelic attachments and aneuploidy after mitotic slippage. Given that mitotic slippage is a well-recognized response to prolonged mitotic arrest[73], mitotic checkpoint overactivation (that is, prolonged inhibition of the APC/C and, consequently, abnormal stabilization of cyclin B1 and securin) could lead to an increased rate of aneuploidy by allowing lagging sister chromatids and merotelic attachments to accumulate. Eventually, mitotic slippage would occur, generating potentially tumorigenic aneuploid progeny. Interestingly, although the molecular events leading to mitotic slippage are still unclear, it is thought to result from the degradation of cyclin B1 in an APC/C-dependent manner despite the activation of the mitotic checkpoint[74].

Therefore, overactivation of the mitotic checkpoint could be a widespread phenomenon in tumours with CIN. *In vitro* studies using nocodazole and monastrol, together with *in vivo*

studies overexpressing mitotic checkpoint genes, favour the hypothesis that aneuploidy in tumours is largely a consequence of the upregulation of mitotic genes and subsequent mitotic checkpoint overactivation. It is of interest that, as mentioned above, several mitotic checkpoint genes are direct E2f targets, indicating that loss of a major tumour suppressor pathway (that is, Rb inhibition of E2f) leads not only to uncontrolled proliferation but is also directly associated with the generation of mitotic CIN[75–79].

Centrosome amplification has also been tightly associated with aneuploidy as a result of aberrant mitoses, and recent studies have shed light on the mechanistic connection between the two. By looking at how cells that have more than two centrosomes and multipolar spindles survive, several cell lines have been found to preferentially cluster multiple centrosomes to two poles, thereby generating a functional bipolar spindle[80,81]. Inhibition of centrosome clustering by short-hairpin RNA (shRNA) targeting of *KIFC1*, which encodes a non-essential kinesin motor protein, led to lethality of multipolar cells[81]. Moreover, clustered bipolar spindles (in tetraploid cells that had more than two centrosomes) were shown to have an increased frequency of merotely, leading to lagging chromosomes and segregation errors[82,83]. Tetraploid cells that had two centrosomes were not observed to have an increased number of lagging chromatids compared with diploid cells, arguing that it is the initial microtubule attachment and subsequent clustering of multiple centrosomes that is conducive to the generation of aneuploidy. An interesting possibility then is that overexpression of *AURKA*, among other genes that regulate mitotic entry and centrosome homeostasis, results in mitotic abnormalities through centrosome amplifications. One can therefore propose that clustering of centrosomes in cells that have multipolar spindles leads to merotelic attachments and that these are conducive to aneuploidy; similar to what is observed during mitotic checkpoint overactivation.

Consequences of CIN

The notion that CIN serves as a tumorigenic driving force has been expanded by a series of observations from mouse models to include the idea that CIN might also be tumour suppressive in certain contexts. In this section we discuss the evidence from mouse models regarding the consequences of CIN at the cellular and organismal levels. These studies underscore the importance of generating mouse models that faithfully recapitulate the biology of human disease to draw physiologically meaningful conclusions.

As noted above, both mitotic checkpoint weakness and mitotic checkpoint overactivation can lead to CIN through different mechanisms. Haploinsufficiency for *Cenpe*[55], *Mad2l1* [REF. 49], *Mad1l1* (REF. 84), *Fzr1* (which encodes CDH1)[5], *Plk4* (REF. 85) and a hypomorphic allele of *Bub1* (REF. 54) all lead to moderate levels of aneuploidy and an increase in the incidence of spontaneous late-onset tumours of lymphoid origin and tumours in some epithelial tissues (especially lung and liver) in mice. This is also the case for the $Cdc20^{AAA}$ mutant, the product of which fails to interact with MAD2; homozygous $Cdc20^{AAA}$ mice are embryonic lethal yet heterozygous adults are tumour prone[56]. Although spontaneous tumour onset does not necessarily result from CIN, as indicated by the lack of such a phenotype in $Bub1b^{+/-}$ (REFS 50,51), $Bub3^{+/-}$ (REFS 52,53) and heterozygous RNA export 1 ($Rae1^{+/-}$)[52] mice, CIN in these cases often increases sensitivity to carcinogen-

induced tumours. Why spontaneous tumour onset differs between the different animal models of CIN is unclear but it does not seem to be related to the degree of CIN[86]. Other mitotic checkpoint-independent functions of these genes could account for these differences between the models but this has yet to be tested[86].

Underscoring the previously mentioned observations from human tumours, spontaneous tumorigenesis is also a consequence of checkpoint overactivation, as seen when MAD2 (REF. 6) or HEC1 (REF. 71) is overexpressed, or in the presence of centrosome amplifications as seen when AURKA is overexpressed[87]. In the case of ubiquitous overexpression of MAD2 or HEC1, spontaneous tumour onset occurs earlier and in a wider range of tissues than that seen for partial loss-of-function mutations. AURKA was only overexpressed in transplanted mammary epithelial cells and so it is unclear whether ubiquitous overexpression of AURKA would show similar phenotypes to MAD2 and HEC1 overexpression in mice. As discussed above, both mitotic checkpoint overactivation and centrosome amplifications lead to lagging chromosomes and merotelic attachments, which, as discussed above, facilitate aneuploidy.

W-CIN and other collateral forms of DNA damage acquired during mitosis, such as chromosome breaks, deletions and amplifications (S-CIN), might together lead to more robust tumour penetrance. Interestingly, gene expression signatures derived from human tumours with CIN have shown that genes involved in DNA damage repair pathways are overexpressed in aneuploid tumours[88]. Overexpression of these genes seems to be necessary for resistance to chemotherapeutic agents that target microtubules, both for chromosomally unstable cell lines and in a subset of ovarian and breast tumours. These results suggest that the DNA damage repair pathway may be activated during the generation of CIN and is required for subsequent viability, although this hypothesis remains to be tested. MAD2 overexpression, which leads to transient mitotic arrest, has been shown to lead not only to W-CIN but also to double-strand breaks, interstitial deletions and amplifications[6]. The prevalence of mitotic checkpoint pathway hyperactivation through the overexpression of MAD2 or other components might therefore explain the common appearance of the DNA damage response in a wide spectrum of aneuploid tumours. Prolonged mitotic arrest through the chemical inhibition of microtubule function also leads to a high incidence of lagging chromatids, merotely and chromosome bridges, all of which could lead to DNA breaks as the cleavage furrow progresses during cytokinesis. Whether DNA damage also occurs in other models of aneuploidy has not been determined, but it might underlie some of the differences in tumour phenotypes observed between different models of CIN.

It is important to note that none of the genetic mechanisms used to generate aneuploidy in animal models results in as rapid an onset in tumorigenesis as seen with activating mutations of classic oncogenes, such as Ras family members[89] and *MYC*[90], or the loss of classic tumour suppressor genes, such as *TP53* (REF. 91) and *RB1* (REF. 92). This could be owing to the fact that the induced genomic instability is sufficient to induce transformation but is held in check by an uncharacterized surveillance mechanism that efficiently destroys transformed cells and persists for many months. Alternatively, low-level genomic instability may require multiple events to first establish the transformed state.

Three studies suggest that low-level aneuploidy such as that generated in the above-mentioned models has detrimental effects on the viability of primary cells. Thompson and Compton[72] studied the effects of CIN generated by transient mitotic checkpoint overactivation using microtubule-stabilizing agents or monastrol on single-cell colonies of two sets of primary cell lines. Single-cell colonies were then analysed by chromosome-specific fluorescence *in situ* hybridization (FISH) to measure aneuploidy. Although mitotic checkpoint overactivation clearly increased the fraction of aneuploid cells in the first few passages, cells in later passages were remarkably euploid. It is still unclear whether the increase in the proportion of euploid cells is a result of a decrease in proliferation rate or increased cell death of aneuploid cells. Nevertheless, it is reasonable to conclude from these studies that aneuploidy is detrimental to the fitness of primary cells.

Williams *et al.*[93] used a different strategy to generate isogenic lines of murine fibroblasts that carried trisomies for chromosomes 1, 13, 16 and 19 in the background of Robertsonian translocations. The decreased growth rates, immortalization rates and metabolic activity in most of the trisomic lines led the authors to conclude that low-level aneuploidy in primary cells has detrimental effects not only on organismal fitness but also on cellular fitness and viability. Although the Robertsonian translocations by themselves had no effect on immortalization times, it is possible that the combination of trisomies and Robertsonian translocations were both required for the observed properties of the cells.

Finally, although inducible MAD2 overexpression in mice leads to the appearance of tumours in a range of different organs, MAD2 overexpression in fibroblasts has a marked negative effect on cellular viability[6]. The overall conclusion from these studies is that in primary cells CIN is detrimental to viability and is therefore selected against. Whether a specific ‘aneuploidy sensor’ is responsible for this impaired fitness or whether it results from an alteration in global transcription remains to be determined. Evidently, this aneuploidy sensor is not 100% efficient, as some trisomies or monosomies are carried to term and, in the cases of Down’s, Turner’s and Klinefelter syndromes, tolerated with few global abnormalities. Nor is this sensor a ubiquitous property of primary cells, as mouse embryonic fibroblasts that lack any Rb family members ($Rb1^{-/-}$, $Rbl1^{-/-}$ (which encodes p107) and $Rbl2^{-/-}$ (which encodes p130)) rapidly tend towards tetraploidy in the first 20 passages[94], although the Rb pathway may be necessary for this sensor. Nevertheless, these findings may explain why mouse models of aneuploidy take so long to develop tumours. Most abnormal mitoses will not generate aneuploid cells that have a substantial growth advantage, and it is only after a particularly long period of time that transformed progeny arise.

A study in 2007 by the Cleveland lab[55] suggested that aneuploidy could both promote and suppress tumorigenesis depending on the tissue and genetic contexts. $Cenpe^{+/-}$ mice developed CIN and spontaneous tumours in a similar pattern to other mitotic checkpoint genes. The evidence for the tumour suppressive role of aneuploidy was a 50% reduction in the incidence of liver tumours and a reduction in DMBA-induced tumours, neither of which were statistically significant and could therefore be due to chance alone. In addition, there was a statistically significant but only slight delay in tumour-free survival in $Cdkn2a^{ARF}$-null mice, which is a common tumour-prone background used to explore tumour suppressor effects. Nonetheless, other reports have since shown similar results. The incidence of small

intestinal tumours was reduced twofold by *Bub1b* haploinsufficiency in the adenomatosis polyposis coli (*Apc*)$^{Min/+}$ mouse model[95]. Remarkably, the incidence of colon tumours in this model was increased tenfold. The Malumbres group[5] showed that, although *Fzr1*$^{+/-}$ animals have increased rates of aneuploidy and an increased incidence of spontaneous tumours compared with wild-type controls, treatment with the carcinogen DMBA results in fewer lung tumours.

If CIN has a tumour suppressive role as a result of excessive genomic damage and subsequent apoptosis or other forms of cell death, one would expect to see such events in the corresponding tissues. Except for the presence of apoptotic cells in the areas surrounding the small intestinal tumours of *Bub1b*$^{+/-}$;*Apc*$^{Min/+}$ mice, no such evidence exists. Perhaps more importantly, it is unclear whether it is CIN itself that contributes to the decreased tumour incidence. The ploidy status of tumours or earlier preneoplastic lesions needs to be examined carefully to draw such a conclusion. This type of analysis is confounded by the difficulty of growing tumour cells *in vitro* for metaphase chromosome counts, and the extrapolation of karyotypes from fibroblasts or lymphocytes from animals that develop intestinal (in the case of *Bub1b*$^{+/-}$;*Apc*$^{Min/+}$ mice) or lung and skin tumours (using DMBA) is not sufficient evidence for aneuploidy in tumour lesions. One way to obtain these data would be to use FISH or array-comparative genomic hybridization (CGH) to determine the extent of aneuploidy in the normal tissues and in the early lesions that arise in these models. However, CGH results are often confounded by the fact that only clonally expanded genomic lesions can be detected from a population of unstable cells and so in these cases only methods that offer single-cell resolution (such as FISH) would identify random CIN.

Finally, the possibility of non-cell-autonomous effects of adjacent tissues or infiltrating bone marrow-derived cells on tumour growth needs to be addressed. It is possible that tumour suppression is a result of non-cell-autonomous effects through which CIN in cells that compose the tumour microenvironment modulates the host response to the primary tumour. Conditional inactivation studies of selected cell types in mice and careful examination of ploidy changes early in the tumorigenic process need to be performed. Such non-cell-autonomous effects could also be responsible for the generation of tumours that result from organism-wide mitotic checkpoint partial loss-of-function or overactivation but, in general, changes in the tumour microenvironment that promote tumorigenesis on their own are much less common than those that lead to tumour suppression.

Ultimately, it is possible and even likely that excessive CIN inhibits cell viability and, as a result, tumour formation. Cell lethality is a consequence of complete loss of the mitotic checkpoint. Nevertheless, much like the case with ionizing radiation, it seems clear that moderate levels of genomic instability can be the evolutionary fuel that generates pro-tumorigenic changes. Most human tumours show clear evidence of CIN and, in these lesions, the tumour suppressive role that this level of genomic instability initially conferred is eventually overcome. The idea that moderate levels of CIN may have tumour suppressive effects remains an important observation in a few mouse models but a more careful analysis will be required to establish whether this is a general principle that is likely to apply to human disease.

Drugging the mitotic checkpoint pathway

Many chemotherapeutic agents result in activation of the mitotic checkpoint. Microtubule-stabilizing drugs (such as taxanes) and depolymerizing drugs (such as vinka alkaloids) are regularly used as mainstay therapy in several solid tumours, often having marked efficacy[96]. Nevertheless, the substantial side effects of these drugs, which result from myelosuppression and neurotoxicity, have spawned a search for newer, more specific drugs that might target cells that have an abnormal mitotic checkpoint. TABLE 2 summarizes some of the classic chemotherapeutic agents that target mitosis and some of the more recently developed anti-mitotic agents in clinical trials. Remarkably, a recent study[65] has shown that the responses of cells to both classical anti-mitotic drugs and newer agents (such as EG5 inhibitors) show substantial variation, not only between different cell lines but also between cells of the same cell line. Irrespective of whether the cancer cell lines studied showed CIN or not, several responses were elicited following exposure to the drug, ranging from death during mitosis to mitotic slippage, death in the following interphase to a second round of mitosis. The authors propose a model in which DNA damage that is incurred during a prolonged mitotic arrest leads to caspase 9-mediated cell death, but that the timing of cell death depends on whether cells remain in mitosis or slip through it following degradation of cyclin B1. These two thresholds, activation of caspase 9 and degradation of cyclin B1, are thought to be responsible for the observed intra- and inter-line variations in drug response[65]. Given the pro-tumorigenic effects of mitotic checkpoint overactivation, it is possible that the use of microtubule drugs that overactivate the mitotic checkpoint might occasionally result in tumour progression after an initial response. In line with these findings, a recent study has shown that preventing mitotic slippage by downregulating CDC20 may increase the sensitivity of tumour cells to microtubule-targeting agents[97], providing an alternative therapeutic window.

The fact that cells with CIN have evolved to survive repeated rounds of mitotic arrests suggests that it might be preferable to inhibit the mitotic checkpoint or the centrosome abnormalities previously described. The MPS1 and AURKA kinases show some promise in this regard, and the cell lethality of mitotic checkpoint inhibition supports this approach although a means of specifically targeting tumour cells is not yet apparent. Targeted drug delivery or perhaps the hypersensitivity of tumour cells addicted to an overactive checkpoint might provide the therapeutic window required for drug efficacy.

In addition, centrosome clustering seems to be a survival mechanism used by cells that might otherwise carry out an abnormal and lethal multipolar mitosis[81]. Centrosome clustering therefore becomes an attractive target that is remarkably specific to cancer cells. Indeed, the normally non-essential kinesin motor protein KIFC1 is required for the viability of extra centrosome-containing cells[81]. It remains unclear, however, how efficient this targeting approach will be given the high rate of escape of cancer cells already observed in cell line analyses.

Future perspectives

The role of CIN in cancer remains filled with questions and some contradictions between the observations that arise from different laboratories and different model organisms. Although it now seems likely that CIN provides the evolutionary fuel to initiate and propagate the transformed state in several solid tumours, the oncogenic pathways that are activated or the tumour suppressor pathways compromised have yet to be elucidated. In addition, although the role of CIN in inhibiting tumour formation in humans is a possibility, the nature of this tumour suppressive role remains poorly defined. We stress that the observations made in model organisms must be viewed in relation to human cancer. The coming years will most likely see new answers to questions, such as when does mitotic CIN arise in human tumours and in which tumour types does it have an important role in growth, progression and/or metastasis? Can cells that have mitotic CIN be targeted efficiently from a therapeutic standpoint? On the 200th anniversary of the birth of Charles Darwin, it is striking that an analysis of evolution and natural selection — in this case in the form of CIN and cancer progression — is at the forefront of our battle against this devastating disease.

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Glossary

Chromosome instability
The inability to maintain a correct chromosome complement after cell division.

Aneuploidy
An abnormal chromosome number.

Mitotic checkpoint
A cell cycle checkpoint that arrests cell division at metaphase until all sister kinetochores are attached to microtubules from opposite spindle poles.

Breakage–fusion–bridge cycles
A process of amplification in which two centromeres of a dicentric chromosome are pulled to opposite poles during mitosis. If the chromosome breaks then the double-stranded breaks persist in the following S-phase and can contribute to translocations or form new dicentric chromosomes that continue the process of instability.

Whole chromosome instability
This describes CIN in terms of abnormal numbers of chromosomes.

Segmental chromosome instability
This describes CIN in terms of structural abnormalities, such as translocations, inversions, interstitial deletions and amplifications.

Kinetochore
The protein complex that assembles around centromeric chromosome regions and is the source of the signal that activates the mitotic checkpoint and the site of spindle fibre attachment.

Spindle pole
The site of origin of microtubule fibres in mitosis. In most cells this site is delineated by the presence of centrosomes that act as microtubule organizing centers.

Cohesin
A protein complex composed of structural maintenance of chromosomes 1A (SMC1A), SMC3, sister chromatid cohesion 1 (SSC1, also known as RAD21) and SSC3 (also known as stromal antigen 1), the function of which is to topologically link sister chromatids before metaphase.

Anaphase promoting complex/cyclosome
A large E3 ubiquitin ligase complex that degrades cyclin B1 and securin once the mitotic checkpoint is satisfied.

Spindle poison
A compound that affects microtubule function and therefore mitotic spindle formation by stabilizing (such as taxanes) or depolymerizing (such as vinka alkaloids or nocodazole) microtubules.

Transformation
A mechanistically defined process in which a primary cell acquires the ability to grow indefinitely *in vitro* (immortalization), form colonies in soft agar (anchorage-independent growth) and form tumour xenografts when implanted intradermally in nude mice.

Microcephaly
An abnormally small head circumference, which usually results from abnormal brain development.

Mosaic aneuploidy
A tissue in which groups of cells contain chromosome complements that differ from those of neighbouring cells.

Merotelic attachment
When a single kinetochore is attached to microtubules from two spindle poles rather than to one pole.

Mitotic slippage
The process by which a cell arrested in mitosis proceeds through anaphase despite an active mitotic checkpoint.

Monastrol
A small molecule inhibitor of the plus-end directed KIF11 kinesin motor, the function of which is required for chromosome segregation in mitosis.

Nocodazole
A chemical inhibitor of microtubule polymerization often used to activate the mitotic checkpoint and therefore arrest cells in the G2/M phase of the cell cycle.

Lagging chromosome
In anaphase, pairs of sister chromatids that remain at the metaphase plate, often as a consequence of merotelic attachment, can be the source of aneuploidy in the resulting daughter cells. This is distinct from a single chromatid that fails to segregate upon disjunction from its sister chromatid.

Chromosome bridge
In anaphase, a chromosome that bridges the two separating daughter nuclei as a result of abnormal attachments.

Robertsonian translocation
A non-reciprocal chromosomal translocation in which two distinct acrocentric chromosomes become fused and share a single centromere.

DMBA-induced tumour
An induced tumour model in mice in which the carcinogen DMBA (7,12-dimethylbenz(a) anthracene) is applied to the skin of 5–7 day old pups. This results in the appearance of skin and lung tumours once animals reach adulthood.

Array-comparative genomic hybridization
A genomic DNA hybridization technique that allows high-resolution analysis of copy number changes between two populations (such as normal versus tumour DNA).

Non-cell-autonomous effect
A phenotypic effect seen in a field of cells that are mediated by cells that are not part of that field, such as the clearance of tumour cells by bone marrow cells or cells of the tumour microenvironment.

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At a glance

- Chromosomal instability (CIN), the inability to correctly segregate sister chromatids during mitosis, provides the evolutionary fuel to initiate and propagate the transformed state of multiple forms of cancer.
- The mitotic checkpoint is seldom lost or weakened in human tumours.
- Mitotic checkpoint overactivation is a more frequent observation in human tumours and is sufficient to generate CIN *in vivo* and *in vitro*. Mitotic checkpoint overactivation results in a prolonged mitosis, abnormal stabilization of cyclin B1 and securin, and an increased incidence of merotelic attachments and lagging chromosomes.
- Many of the key regulators of the mitotic checkpoint are downstream targets of the Rb tumour suppressor pathway and are therefore upregulated in most human tumours.
- The consequences of CIN are manifold and context-dependent. Although CIN can initiate tumour formation in many mouse models, under some conditions it decreases cellular fitness, providing a potential tumour suppressor effect. This effect is nevertheless often overcome, giving rise to the karyotypic complexity observed in advanced tumours.
- Mitotic checkpoint overactivation could prove effective as a novel therapeutic target as mitotic checkpoint loss is incompatible with cellular viability.

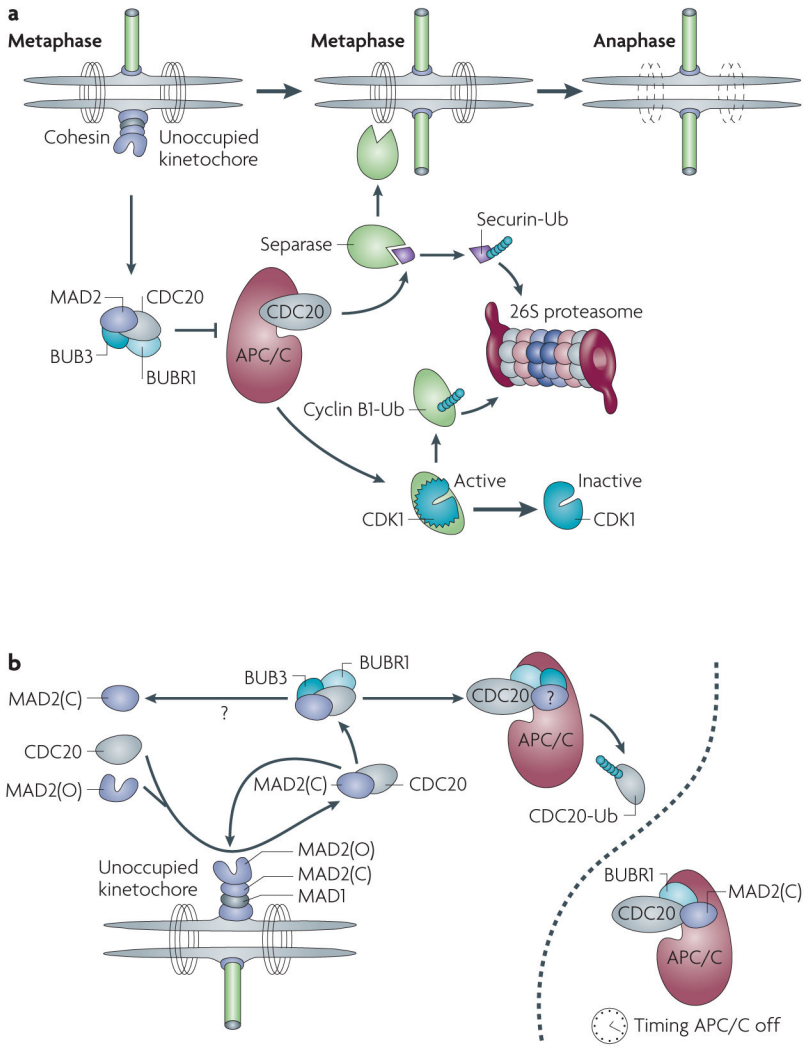

Figure 1. The mitotic checkpoint

a | Outline of the mitotic checkpoint. An unattached kinetochore is shown on the left with the inner complex in purple, MAD1 in grey and MAD2 in its open and closed forms in purple. The mitotic checkpoint complex (MCC) is shown to inhibit the anaphase promoting complex/cyclosome (APC/C), which after attachment of the last kinetochore is activated and ubiquitylates securin and cyclin B1. More details of this pathway are described in the main text. **b** | The amplification of the unoccupied kinetochore signal is thought to depend on the conversion of MAD2 open complexes (MAD2(O)) to closed complexes (MAD2(C)) that bind to cell division cycle 20 (CDC20) and deliver it to the APC/C for ubiquitylation (Ub). The nature of the MCC is still debated, as indicated by question marks. A separate APC/C is shown to indicate its role in timing, independent of the kinetochore-derived signal. CDK1, cyclin-dependent kinase 1.

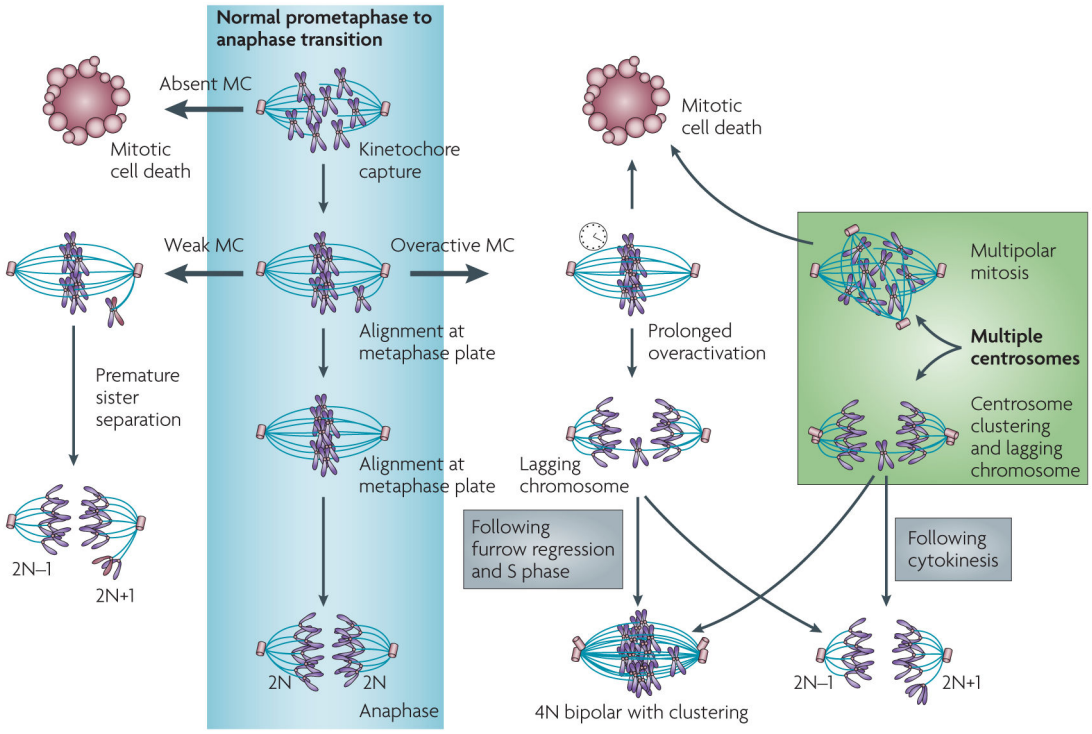

Figure 2. Multiple mechanisms leading to aneuploidy

The normal mitotic checkpoint (MC) events from prometaphase to anaphase are shown in the centre. An absent checkpoint leads to mitotic cell death. A weak checkpoint (left) leads to premature sister chromatid separation and near-diploid aneuploidy. An overactive checkpoint (right) can lead either to mitotic cell death or lagging chromosomes and subsequent near-diploid aneuploidy or tetraploidy. Multiple centrosomes can have similar consequences to an overactive checkpoint. A multipolar mitosis leads to cell death unless centrosomes cluster, in which case the likelihood of lagging chromosomes is high.

Table 1

Cancer models of CIN

Gene	Cancer-associated mutation	Altered expression in tumours	Model	Tumour-associated phenotype *in vivo*
AURKA	Amplifications in different types of human cancer[106–108]	Overexpressed in breast[109], colorectal[108,110], ovarian[111], pancreatic[112], gastric[113], oesophageal, bladder[107], cervical[114], and head and neck cancer[115]	*Aurka*$^{+/-}$	Heterozygous mice develop lymphomas, hepatomas, lung adenocarcinomas and squamous cell carcinomas[116]
			Cre-CAT-*Aurka*; WAP-Cre	Overexpression induces mitotic abnormalities and mammary gland hyperplasia[87]
			MMTV-*Aurka*	Overexpression induces genetic instability preceeding mammary tumour formation[40]
			Aurka$^{f/f}$	No tumour phenotype reported[117]
AURKB		Overexpressed in astrocytomas[118], seminomas[119], ependymomas[120], prostate cancer[121] and non-small-cell lung carcinomas[122]; predictive factor for recurrence of hepatocellular carcinomas[123]	No spontaneous models	Overexpression of a wild-type form or a non-degradable form in murine epithelial cells generates tumours in nude mice[124]
BUB1	Mutated in colon, lung tumours[125] and very low frequency of mutation in pancreatic cancer cells[126]; promoter hypermethylation in colon carcinoma[127,128]	Reduced expression in AML[128]; overexpressed in breast cancer and cell lines[129], in non-endometrioid endometrial carcinoma[130], gastric cancer[131], clear cell kidney carcinoma[132], and thyroid carcinoma[133]; mutated in colon cancer cell lines and corresponding human samples[134]	*Bub1*$^{+/-}$, *Bub1*$^{H/H}$, *Bub1*$^{-/H}$	Heterozygous mice are more susceptible to DMBA-induced lung tumours; *Bub1*$^{H/H}$ mice develop spontaneous sarcomas and hepatocellular carcinomas; *Bub1*$^{-/H}$ mice have an increased incidence of lymphomas, lung adenomas and sarcomas[54]
			Bub1$^{\Delta 2-3/\Delta 2-3}$	76% of mice (expressing hypomorphic BUB1 mutant that lacks exons 2 and 3) develop spontaneous lung and liver tumours[135]
BUB1B (encodes BUBR1)	Promoter hypermethylation in colon carcinoma[127]	Overexpressed in breast cancer and cell lines[129], in gastric cancer[131], clear cell kidney carcinoma[132] and thyroid carcinoma[133]	*Bub1b*$^{+/-}$	No spontaneous tumours[50]; microadenomas and tubular adenomas of the colon, lung adenocarcinomas and liver neoplasms after AOM treatment[51]
			Bub1b$^{H/H}$	No spontaneous tumours[50]; DMBA-treated mice are prone to lung tumours[136]
BUB3		Overexpressed in primary breast cancer[129] and gastric carcinomas[131]	*Bub3*$^{+/-}$	Not determined[53]; no cancer predisposition[137]; no statistically significant increase in lung tumours after DMBA treatment[52]

Gene	Cancer-associated mutation	Altered expression in tumours	Model	Tumour-associated phenotype *in vivo*
			Bub3$^{+/-}$;*Trp53*$^{+/-}$ and *Bub3*$^{+/-}$;*Rb1*$^{+/-}$	No differences in the number or rate of tumours compared with single mutants[137]
			Bub3$^{+/-}$;*Rae1*$^{+/-}$	Increased incidence of lung tumours after DMBA treatment[52]
CDC20		Overexpressed in oral squamous cell carcinoma cell lines and in head and neck tumours[138], pancreatic[139], breast[129], gastric[140], ovarian cancer[141], gliomas[142] and in early-stage lung adenocarcinoma[143]	*Cdc20*$^{+/AAA}$ (mutant cannot be inhibited by MAD2)	Spontaneous development of lymphomas and hepatomas at 24 months of age[56]
FZR1 (encodes CDH1)		Reduced expression in breast, colon and rectal tissue microarrays[144]; overexpressed in seminoma, neuroblasto ma, medulloblastoma, oesophageal adenoma, colon cancer, lung cancer, breast cancer and lymphoma[145]	*Fzr1*$^{+/-}$	25% of *Fzr1*$^{+/-}$ mice develop epithelial neoplasias, such as adenocarcinoma and fibroadenoma of the mammary gland, lung, liver, kidney, testis and sebaceous gland tumours at long latencies[5]
CENPE		Low CENPE levels in benign tumours and increased levels in malignant pituitary neoplasias[146]	*Cenpe*$^{+/-}$	10% of *Cenpe*$^{+/-}$ mice develop lymphomas and 10% develop lung adenomas with very long latencies[147]; decreases in the incidence of liver tumours and DMBA-induced tumours were reported for *Cenpe*$^{+/-}$ animals but neither was statistically significant
			Cenpe$^{+/-}$;*Cdkn2a*$^{ARF-/-}$	Increased survival relative to single mutants[148]
CCNB1		Overexpressed in pulmonary adenocarcinoma[149], non-small-cell lung cancer[150,151], gastrointestinal stromal tumours[152], oesophageal squamous cell carcinoma[153,154], renal cell carcinoma[155] and breast cancer[156]; correlates with poor survival in breast cancer[157]	*Ccnb1*$^{-/-}$	Die *in utero*[158]

Gene	Cancer-associated mutation	Altered expression in tumours	Model	Tumour-associated phenotype *in vivo*
NDC80 (encodes HEC1)		Overexpressed in lung cancer and correlates with poor prognosis[159]; overexpressed in lung, liver and brain tumours[160]	CMV-TetO*Ndc80*	40% of mice develop tumours (lung and hepatocellular adenomas and sarcomas)[71]
MAD1L1 (encodes MAD1)	Mutated in cancer cells from lymphoid, pancreas, prostate, breast and lung tissues[161,162]	Reduced expression in human gastric cancer, poorly differentiated tumours[163,164] and hepatocellular carcinoma[165]; loss of MAD1 is implicated in tumour recurrence	*Mad1l1*$^{+/-}$	19% of mice develop spontaneous tumours at 18 months of age[84]
			Mad1l1$^{+/-}$;*Mad2l1*$^{+/-}$; *Trp53*$^{+/-}$	Increased tumour frequency[166]
MAD2L1 (encodes MAD2)	Rare mutations in bladder and breast cancer cells[167,168]	Overexpressed in several tumour types[68], such as malignant lymphoma[169], liver cancer[170], lung cancer[171,172], soft tissue sarcoma[173], hepatocellular carcinoma, gastric cancer[174] and colorectal carcinoma[175]	*Mad2l1*$^{+/-}$	27% develop lung tumours at 18 months of age[104]
			Mad2l1$^{+/-}$;*Trp53*$^{+/-}$	Increased tumour frequency[166]
			CMV-TetO*Mad2l1*	MAD2 overexpression induces a wide range of neoplasias and accelerates tumorigenesis induced by MYC[6].
			TetO*Mad2l1*;TetO*Kras; Scgb1a1*-rtTA	MAD2 overexpression accelerates lung tumorigenesis induced by mutant KRAS[200]
PTTG1 (encodes securin)		Overexpressed in pituitary[37], pancreatic ductal carcinoma[176], lung[177,178], glioma[179], hepatocellular carcinoma[180,181], prostate[182], ovarian[183], colorectal[184], thyroid[185] cancers and multiple myeloma[186]; also a marker of metastatic tumours[187,188]	*Pttg1*$^{-/-}$	No tumours[189]
			Pttg1$^{-/-}$;*Rb1*$^{+/-}$	Decrease in pituitary tumours relative to *Rb1*$^{+/-}$ (REF. 190)
			Cga-Pttg1	Hyperplasia and microadenomas of the pituitary[191]
			Cga-Pttg1;Rb1$^{+/-}$	Increased frequency of anterior lobe tumours[192]
PLK1	Specific mutations in some cell lines alter protein stability[193]	Upregulated in breast[194], oesophageal[194], lung[195], colorectal cancer[196] and anaplastic thyroid carcinoma[197]	*Plk1*$^{+/-}$	27% develop lymphomas, lung adenocarcinomas, squamous cell carcinomas, and ovarian sarcomas[198]
			Plk1$^{+/-}$;*Trp53*$^{-/-}$	Higher frequency of tumours relative to single mutants[198]
PLK4	Loss of heterozygosity in hepatomas[85]	Aberrant expression in colorectal cancer[196]	*Plk4*$^{+/-}$	Increased frequency of hepatocellular and lung carcinomas[85]

AML, acute myeloid leukaemia; AOM, azoxymethane; *AURKA*, aurora kinase A; *AURKB*, aurora kinase B; CAT, catalase; *CCNB1*, cyclin B1; *CDC20*, cell division cycle 20; *CENPE*, centromere protein E; Cga, glycoprotein hormones, alpha subunit (also known as αGSU); CMV, cytomegalovirus; DMBA, 7,12-dimethylbenz(a)anthracene; H, hypomorphic allele; MMTV, mouse mammary tumour virus; PLK, polo-like kinase;

PTTG1, pituitary tumour-transforming gene 1; *Rae1*, RNA export 1; *Scgb1a1*, secretoglobin, family 1A, member 1 (also known as CCSP); Tet, tetracycline; WAP, whey acidic protein.

Table 2

Drugs that target mitosis *

Mechanism of action or target	Examples of drugs	Approved indications	Clinical trial stage	Company (clinical trials.gov identifier)
Microtubule stabilization	Docetaxel	Breast, prostate, non-small-cell lung cancer, gastric cancer, head and neck cancer	FDA approved	
	Paclitaxel	Ovarian cancer, breast cancer, non-small-cell lung cancer and Kaposi's sarcoma	FDA approved	
Microtubule depolymerization	Vinblastine	Hodgkin's and non-Hodgkin's lymphoma, mycosis fungoides, testicular cancer and Kaposi's sarcoma	FDA approved	
	Vincristine	Leukaemias, Hodgkin's and non-Hodgkin's lymphoma, neuroblastoma, rhabdomyosarcoma, Wilms' tumour and Kaposi's sarcoma	FDA approved	
	Vinorelbine	Non-small-cell lung cancer	FDA approved	
KIF11 kinesin inhibitors	Ispinesib	ND	Phase I/II in metastatic breast cancer, lymphoma and multiple other solid tumours	Cytokinetics (NCT00607841) —breast cancer
	SB-743921	ND	Phase I in solid tumours and Phase II in non-Hodgkin's lymphoma	GlaxoSmithKline (NCT00136513) and Cytokinetics (NCT00343564)
	MK0731	ND	Phase I in solid tumours	Merck (NCT00104364)
	ARRY-520	ND	Phase I/II in advanced leukaemia and multiple myeloma	Array BioPharma (NCT00637052 and NCT00821249)
	AZD4877	ND	Phase II in advanced bladder cancer	AstraZeneca (NCT00661609)
Aurora kinase inhibitors	MLN8237	ND	Phase II in AML, ALL, ovarian cancer and non-Hodgkin's lymphoma	Millenium Pharmaceuticals (NCT00830518, NCT00739427, NCT00853307 and NCT00807495)
	AT9283	ND	Phase I and II in leukaemias	Astex Therapeutics (NCT00522990)
	AZD1152	ND	Phase I and II in AML	AstraZeneca (NCT00952588)
CENPE inhibitor	GSK923295	ND	Phase I in refractory cancer	GlaxoSmithKline (NCT00504790)
PLK inhibitors	BI 2536	ND	Phase I in non-Hodgkin's lymphoma	Boehringer Ingelheim (NCT00243087)

Mechanism of action or target	Examples of drugs	Approved indications	Clinical trial stage	Company (clinical trials.gov identifier)
	ON 01910	ND	Phase II in ovarian cancer and myelodysplastic syndrome	Onconova (NCT00856791 and NCT00906334)
CDC2 inhibitor	P276-00	ND	Phase II in malignant melanoma	Piramal Life Sciences (NCT00835419)

*Adapted and modified from REF. 199. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CDC2, cell division cycle 2 (also known as CDK1); CENPE, centromere protein E; FDA, US Food and Drug Administration; KIF11, kinesin family member 11 (also known as EG5); ND, not determined; PLK, polo-like kinase.