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REVIEW

Enhanced genomic instabilities caused by deregulated microtubule dynamics and chromosome segregation: a perspective from genetic studies in mice

Chinthalapally V.Rao*, Hiroshi Y.Yamada, Yixin Yao[1] and Wei Dai[1]

Department of Medicine, Hematology/Oncology Section, University of Oklahoma Cancer Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA and [1]Department of Environmental Medicine, New York University School of Medicine, Tuxedo, NY 10987, USA

*To whom correspondence should be addressed. Tel: +1-405-271-3224; Fax: +1-405-271-3225;
Email: cv-rao@ouhsc.edu

Aneuploidy is defined as numerical abnormalities of chromosomes and is frequently (>90%) present in solid tumors. In general, tumor cells become increasingly aneuploid with tumor progression. It has been proposed that enhanced genomic instability at least contributes significantly to, if not requires, tumor progression. Two major modes for genomic instability are microsatellite instability (MIN) and chromosome instability (CIN). MIN is associated with DNA-level defects (e.g. mismatch repair defects), and CIN is associated with mitotic errors such as chromosome mis-segregation. The mitotic spindle assembly checkpoint (SAC) ensures that cells with defective mitotic spindles or defective interaction between the spindles and kinetochores do not initiate chromosomal segregation during mitosis. Thus, the SAC functions to protect the cell from chromosome mis-segregation and anueploidy during cell division. A loss of the SAC function results in gross aneuploidy, a condition from which cells with an advantage for proliferation will be selected. During the past several years, a flurry of genetic studies in mice and humans strongly support the notion that an impaired SAC causes enhanced genomic instabilities and tumor development. This review article summarizes the roles of key spindle checkpoint proteins {i.e. Mad1/Mad1L1, Mad2/Mad2L1, BubR1/Bub1B, Bub3/Bub3 [conventional protein name (yeast or human)/mouse protein name]} and the modulators (i.e. Chfr/Chfr, Rae1/Rae1, Nup98/Nup98, Cenp-E/CenpE, Apc/Apc) in genomic stability and suppression of tumor development, with a focus on information from genetically engineered mouse model systems. Further elucidation of molecular mechanisms of the SAC signaling has the potential for identifying new targets for rational anticancer drug design.

Introduction

The cell division cycle refers to the period of time between the formation of a cell (the mother cell) and the time when the cell divides to form two daughter cells. The progress of cell cycle events is tightly regulated to maintain the genetic integrity of the cell and to correctly pass genetic information to daughter cells. How cells regulate the integrity of the cell cycle and the genome has been a major subject for research. Extensive research in the past two decades has uncovered surveillance mechanisms commonly referred to as checkpoints and given clearer understanding in maintenance mechanisms for genomic stability.

Two major modes for genomic instability have been described: microsatellite instability (MIN) and chromosome instability (CIN). Microsatellites are stretches of repeated sequences of DNA. Although there are considerable variations in the length among individuals, the length remains relatively stable in the individual under normal circumstances. The appearance of abnormally long or short microsatellites in an individual's DNA is referred to as MIN. MIN is associated with a defect in repairing DNA damage. Broken or translocated chromosomes can arise from MIN condition. In cases of human colorectal cancer, ∼15% of tumors show MIN phenotype. CIN is observed in 85% of colorectal cancers and is defined as abnormalities in chromosome number, such as extra chromosome or missing chromosome. CIN is associated with mitotic errors that lead to mis-segregation of chromosomes. This review focuses on the issues related to CIN and the roles of a set of mitotic regulatory proteins, especially on the spindle assembly checkpoint (SAC) proteins, in maintenance of genomic stability and prevention of cancer.

Accurate chromosome segregation during mitosis is one of the most fundamental processes that allow cells to faithfully transmit their genetic information to daughter cells. Failures in the maintenance of genetic stability during mitosis can lead to cell death or CIN and can significantly contribute to malignant transformation. In fact, cancer cells frequently exhibit numerical abnormalities in chromosomes (aneuploidy). Mis-segregation of chromosomes may result from various causes, including SAC defects, abnormal centrosome formation, impairments in attachment of spindle microtubules to kinetochores and failure of cytokinesis. A series of mammalian genetic studies confirm that the untimely initiation of anaphase due to SAC failure or deregulated activities of anaphase promoting complex/cyclosome (APC/C) predisposes mammalian cells to genomic instability and neoplastic transformation.

This mini review focuses on information from several genetically engineered mouse model systems with regard to the importance of SAC components in suppression of CIN and development of common cancers in the animals, and the information is relevant to human cancers. Compared with heterogenous human cancers, the genetically defined model system provides more interpretable results. Generation of such mouse model system is a part of translational efforts from cell biology to preclinical animal models and has high merits. However, due to the considerable time, cost and efforts required in generating such mouse models, not all SAC or the modulator genes are covered by translational efforts or the results are yet to be published to date. We will review current results regarding four core SAC components (Mad2, Mad1, BubR1 and Bub3), four modulators (Chfr, Rae1, Nup98 and CenpE) and one tumor suppressor (Apc) (Table I). The potential uses of these transgenic knockout mice in the discovery of new compounds effective for cancer treatment are also discussed.

SAC components in the regulation of chromosome stability

To ensue that accurate chromosome segregation is maintained during mitosis, cells have evolved a mechanism termed the SAC or spindle checkpoint. At this checkpoint, the integrity of microtubules and the completion of chromosome alignment with spindle microtubules are monitored (see reviews in refs 1–3). Due to the presence of this surveillance mechanism, normal cells will not proceed to anaphase as long as chromosomes remain unattached to mitotic spindles, and the cells are given time to resolve issues. Early genetic screening approaches have identified at least seven genes that are required for mitotic checkpoint function in yeast (4–6). These include *BUB1*, *BUB2* and *BUB3* (4), *MAD1*, *MAD2*, *MAD3* (5) and *MPS1* (6). Extensive studies in the past decade have demonstrated that the spindle checkpoint is highly conserved and the orthologs are found in mammals (7–11). In addition to orthologs of *Bub* and *Mad* family members (core components of the SAC), several additional genes, such as those

Abbreviations: Apc, adenomatous polyposis coli; APC/C, anaphase-promoting complex/cyclosome; Cenp-E, Centromere protein E; CIN, chromosome instability; MEF, mouse embryonic fibroblast; MIN, microsatellite instability; SAC, spindle assembly checkpoint; tet, tetracycline.

for *Zw10*, *Rod*, *Cenp-E*, *Hec1*, *Rae1* and *Nup98*, also contribute to spindle checkpoint function in mammalian cells (12–15) (Figure 1), (reviewed in refs 16,17). How these genes function in mitosis continues to be a major research subject in the cell cycle/mitosis research field.

Mad2 (human MAD2L1) Human *Mad2* (*Mad2L1*) was the first mammalian gene of the spindle checkpoint pathway to be cloned and characterized and is highly conserved both structurally and functionally (3). The Mad2 protein is localized at kinetochores after chromosome condensation and before anaphase (18) and functions as a key component in mediating the activation of the spindle checkpoint (3). Mouse embryonic blastocysts lacking *Mad2* are able to grow until about embryonic day 5.5 (E5.5). These cells then quickly succumb to apoptosis due to severe chromosome mis-segregation caused by SAC impairment (19). In fact, mice with homozygous deletion at the *Mad2* locus die *in utero* (19). A detailed examination reveals that $Mad2^{-/-}$ embryos are smaller than control littermates and exhibit positive TUNEL (Terminal deoxynucleotydyl transferase dUTP Nick End Labeling) staining (19), indicating that *Mad2* null embryos undergo apoptosis.

A separate study shows that the deletion of one allele of *Mad2* causes defects in the spindle checkpoint, which is manifested as precocious separation of sister chromatids in the presence of microtubule poisons and an enhanced rate of chromosomal mis-segregation (20). As *Mad2* null mice are non-viable, $Mad2^{+/-}$ mice were examined for developmental abnormalities as well as tumor development. $Mad2^{+/-}$ mice developed lung adenocarcinomas at an enhanced rate (>27%) compared with that of control littermates after a long latency (20). Interestingly, the rate of lymphomas in both control and $Mad2^{+/-}$ mice is unaffected by the genetic background. It remains unclear why the lung is more susceptible to the development of cancer in *Mad2*-deficient mice.

Another direction to test the role of Mad2 in tumorigenesis *in vivo* is to construct mice that overexpress Mad2 (21). Mad2 overexpression is observed in a variety of cancers (22–24). To investigate the effects of Mad2 overexpression, Sotillo *et al.* (21) generated mice carrying a tetracycline (tet)-inducible or tet-repressible *Mad2* gene. The use of tet-inducible promoter allowed Mad2 overexpression with tet analog doxycycline-containing diet *in vivo*. Overexpression of Mad2 resulted in prolonged mitosis, elevated mitotic errors and the production of polyploid cells, indicating that Mad2 overproduction can actually produce genomic instability. Fifty percent of the Mad2-overexpressing mice died by 75 weeks, albeit no death in controls. The Mad2-overexpressing mice showed a wide spectrum of tumors including hepatoma, hepatocellular carcinoma, lung ademonas, fibrosarcomas and lymphomas. Use of the human ONCOMINE microarray database showed that Mad2 overexpression is observed in human tumors overlapped with the cancer types found in the mice, which provides further validation. Interestingly, only transient Mad2 overexpression was sufficient for tumor formation, and shutting down Mad2 expression after tumor formation did not result in shrinkage of existing tumor. Thus, unlike a classical oncogene, overexpression of Mad2 is not required for tumor maintenance (21).

Mad1 (human Mad1L1) Mad1 and Mad2 directly interact and the interaction is essential in Mad2 conformation change, an important signal dissemination mechanism of the spindle checkpoint (25,26). Thus, together they define a branch in spindle checkpoint signaling (27). As in Mad2, mice with Mad1 homozygous deletion ($Mad1^{-/-}$) are non-viable (28). Mice with *Mad1* heterozygous deletion ($Mad1^{+/-}$) (haploinsufficiency) are viable; however, $Mad1^{+/-}$mice develop a variety of tumors (hepatocellular carcinoma, rhabdomyosarcoma, osteosarcoma, hemangiosarcoma and uterine sarcoma) with a 2-fold higher incidence compared with control. Moreover, microtubule inhibitor vincristine treatment resulted in neoplasia in 42% of the $Mad1^{-/+}$ mice, but not in wild-type control, suggesting that the wild-type amount of Mad1 functions to prevent neoplasia provoked by microtubule inhibitor. Intriguingly, when $Mad1^{+/-}$ and $Mad2^{+/-}$ are combined at the cellular level [$Mad1^{+/-}$ $Mad2^{+/-}$ mouse embryonic fibroblast (MEF)], the spindle checkpoint function was further compromised and they exhibited a higher rate of aneuploidy and tumorigenic ability compared with $Mad2^{+/-}$ or $Mad1^{+/-}$ cells. The result suggests that although Mad1 and Mad2 function together, there may be some non-overlapping functions unique to Mad1 or Mad2.

BubR1 (human Bub1B) BubR1 functions as a key component in spindle checkpoint activation, during which it is extensively phosphorylated (9). BubR1 interacts directly with Cdc20 (29), an activator of the APC/C, thereby inhibiting the activity of APC/C^{Cdc20}. Hyperphosphorylated BubR1 and other components of the checkpoint machinery including Bub1, Bub3, Mad1, Mad2 and Centromere protein E (Cenp-E) are associated with unattached kinetochores (30). Although BubR1 and Mad2 appear to function in the same signaling pathway after spindle checkpoint activation, BubR1 is a much more potent inhibitor of APC/C than Mad2 (31). Mutations in BubR1 were detected in human colon cancers (7).

In order to study its physiological function, two independent groups investigated the *in vivo* function of BubR1 via the generation of mutant mice (32–34). Similar to that of *Mad2* null mice, *BubR1* null mice are also embryonically lethal (32–34). *BubR1* haploinsufficiency results in an increase in the number of splenic megakaryocytes, which appears to be associated with an elevated level of megakaryocytic, but not erythrocytic, progenitors in bone marrow cells (32). Consistently, $BubR1^{+/-}$ murine embryonic fibroblast cells also contain a large number of polyploid cells, frequently accompanied by micronuclei (32). These observations suggest that deregulated spindle checkpoint action due to inactivation of BubR1 may impair nuclear division or cytokinesis, resulting in the formation of polyploid cells. *BubR1* insufficiency also causes infertility as well as phenotypes characteristic of early aging (33). Specifically, mutant mice with reduced levels of BubR1 expression develop symptoms including cachectic dwarfism, cataracts, lordokyphosis (hunchback spine), loss of subcutaneous fat and reduced wound healing, eventually leading to a shortened lifespan (33). Development of these phenotypes in *BubR1*-deficient mice is associated with a compromised spindle checkpoint because cells from these mice become progressively aneuploid (33). Consistently, MEF cells with graded reduction of BubR1 expression also become aneuploid and senescent (33), supporting the notion that BubR1 may have a function in the control of aging.

Given the major function of BubR1 in regulating chromosomal segregation, it is anticipated that *BubR1*-deficiency would lead to mitotic progression with compromised spindle checkpoint. In fact, *BubR1*-deficient cells are defective in mitotic arrest in the presence of a microtubule-depolymerizing agent nocodazole (34). $BubR1^{+/-}$ mice are prone to develop both colon and lung adenocarcinomas upon carcinogen azoxymethane treatment (35). Moreover, in the $Apc^{Min/+}$ genetic background, *BubR1* haploinsufficiency causes premature separation of sister chromatids, genomic instability and development of spontaneous colonic tumors (35). Whereas $Apc^{Min/+}$ mice develop less than one tumor per mouse (on average), mice heterozygous for *BubR1* and *Apc* mutant alleles develop more than four spontaneous colonic tumors. In addition, tumors from $BubR1^{+/-}Apc^{Min/+}$ mice are highly malignant compared with those from $Apc^{Min/+}$ mice (35). These results indicate that haploinsufficiency of *Apc* and *BubR1* results in a significantly accelerated rate of development, as well as progression of colon cancer compared with that of mice with single gene deficiency. This study thus suggests that *BubR1* and *Apc* functionally interact in the regulation of chromosomal segregation and suppression of genomic instability. The importance of BubR1 in the maintenance of chromosomal stability and suppression of cancer is further supported by a human study (36). A systematic study of five families with mosaic-variegated aneuploidy, a recessive condition characterized by mosaic aneuploidies, identifies missense or truncating mutations in *BubR1*. Individuals with mosaic-variegated aneuploidy are predisposed to develop childhood cancer including rhabdomyosarcoma, Wilms tumor and leukemia (36). This study is the first to associate germline mutations in a spindle checkpoint gene with human cancer, thus strongly supporting a causal connection between chromosomal mis-segregation, aneuploidy and malignant transformation.

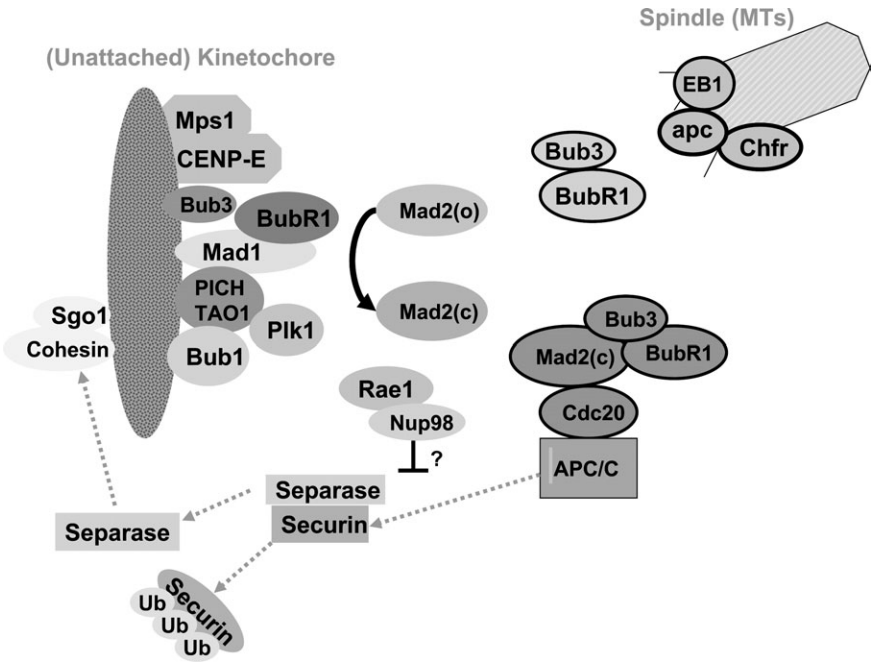

Fig. 1. Regulations on mitotic metaphase–anaphase transition through SAC and APC/C. Mitotic unattached kinetochores are critical structures in mitotic regulations. They serve as a scaffold for mitotic regulatory signaling proteins including SAC proteins, and the enriched localization of signaling proteins is thought to be important for proper SAC function. Encircled by bold line are the proteins discussed in text. Mps1 is an SAC kinase. CENP-E is a mitotic motor whose inactivation abrogates SAC function. Bub3 binds to BubR1 kinase, both are core SAC components. Mad1 is an SAC component and catalytically converts Mad2 three dimensional structure from open [Mad2(o)] to closed [Mad2(c)] form. The closed form Mad2(c) makes a part of the SAC inhibitory complex. The SAC inhibitory complex binds to a ubiquitin ligase APC/C and inhibits the activity. Once SAC-mediated inhibition is released, the APC/C polyubiquitylates its mitotic targets, e.g. Cyclin B and Securin, and leads them to proteasome-dependent degradation. Separase is a protease that degrades cohesins. Cohesins hold sister chromatids together. Sgo1 protects cohesins from premature degradation through physical association. TAO1 (One thousand and one amino acids) is an SAC kinase that aids Mad1–Mad2 interaction. Plk1 is a mitotic kinase whose inactivation abrogates Mad1 and Mad2 localization to kinetochores. Plk 1 interacting checkpoint helicase (PICH) is required for Mad1–Mad2 interaction. Chfr localizes on mitotic spindles. Apc interacts with EB1 and localizes on plus end of microtubules. Rae1–Nup98 complex, which is a part of nuclear pore complex during interphase and breaks into subcomplex during mitosis, inhibits premature securin degradation through interaction with APC/C during early mitosis.

Bub3 Human *Bub3* encodes a protein with four WD (tryptophan and aspartic acid) repeats, motifs involved in protein–protein interaction. Similar to Bub1, Bub3 also localizes to kinetochores before chromosome alignment (37). In fact, Bub3 interacts with Bub1, and it is proposed that one role of Bub3 is to facilitate kinetochore localization of Bub1 (37). Bub3 is also part of mitotic checkpoint complex, which functions to inactivate Cdc20 upon activation of the spindle checkpoint (38).

Bub3 is essential for early embryonic development. Mice with biallelic mutations are embryonically lethal (39). *Bub3* null embryos appear to be normal up to embryonic day 3.5, but begin to exhibit nuclear abnormalities including the formation of micronuclei and a chromatin bridge as well as the presence of lagging chromosomes (39). *Bub3* null embryonic cells are unable to arrest in metaphase when they are treated with a microtubule-disrupting agent; haploinsufficiency of *Bub3* results in a spindle checkpoint defect associated with chromosomal mis-segregation and formation of aneuploidy (39). Compound mutations in both Bub3 and Rae1 (a protein sharing sequence homology with Bub3) lead to much enhanced rates of premature separation of sister chromatids and chromosome mis-segregation compared with cells with mutations in a single gene (40). Upon treatment with dimethylbenzanthrene, a lung-specific carcinogen, $Bub3^{+/-}$ mutant mice develop lung tumors at an enhanced rate compared with that of wild-type littermates; moreover, the incidence of lung tumors in $Bub3^{+/-}Rae1^{+/-}$ compound mutant mice is further increased (40), suggesting that there is a synergy between *Bub3* and *Rae1*.

Modulators of the spindle checkpoint in chromosomal stability and tumorigenesis

Chfr *Chfr* was first identified as a gene frequently mutated or inactivated through hypermethylation in a variety of cancers (41,42). Chfr coordinates an early mitotic progression by delaying chromosome condensation in response to a mitotic stress (42). Inactivation of *Chfr* is associated with defects in the spindle checkpoint function; cancer cells lacking *Chfr* exhibit sensitivity to microtubule stressors such as docetaxel or paclitaxel (43). *Chfr* encodes a protein with fork head associated and RING domains and has E3 ubiquitin ligase activity. Recent studies indicate that Aurora A kinase is a key mitotic protein whose level is negatively controlled by *Chfr* (44). Given that Aurora A is overexpressed or amplified in a variety of tumor cell lines and primary tumor specimens, *Chfr* may regulate chromosomal stability by controlling the level of Aurora A during mitotic progression.

A recent mouse genetic study reveals that *Chfr* does function as a tumor suppressor *in vivo* (44). *Chfr* null mice are viable and have no apparent developmental defects, indicating that it is not essential for cell cycle progression during embryonic development (44). However, by ~9 months, a significant number of mice with homozygous deletion of the *Chfr* locus develop invasive lymphoma. $Chfr^{+/-}$ mice also develop lymphoma at an enhanced rate compared with that of wild-type littermates (44). Between 9 and 18 months, many mice develop solid tumors of the lung, liver and gastrointestinal tract (44). The significantly higher tumor incidence in *Chfr* null mice strongly suggests that this gene plays an important role in suppressing tumor formation, probably through controlling chromosomal stability. Indeed, $Chfr^{-/-}$ MEFs exhibit a tendency toward becoming polyploidy. They also have various manifestations of mitotic errors including the presence of lagging chromosomes, chromosome mis-segregation and cytokinesis failure (44).

Rae1 and Nup98 Rae1 (RNA export 1) was originally identified as a component of the nuclear pore complexes, which are large protein assemblies embedded in the nuclear envelope (45). Subsequent studies indicate that Rae1 is a microtubule-associated protein capable of binding directly to importin-β and that it plays a role in positively regulating

Table I. Spindle checkpoint-related mutations and tumorigenesis

Gene (mouse/human)	Genotype	Phenotype	References
Mad2 (*Mad2L1/Mad2L1*)	−/−	Embryonic lethal	(19)
	+/−	Lung adenocarcinoma, prone to polyploid	(20)
	Overexpression	Increase in hepatoma, hepatocellular carcinoma, lung adenoma, fiblosarcoma, lymphoma	(21)
Mad1 (*Mad1/Mad1L1*)	−/−	Embryonic lethal	(28)
	+/−	Increase in hepatocellular carcinoma, rhabdomyosarcoma, osteosarcoma, hemangiosarcoma, uterine sarcoma	(28)
BubR1 (*BubR1/Bub1B*)	−/−	Embryonic lethal	(33,34)
	+/−	Increase in megakaryocytic cells	(32)
		Prone to polyploid	(33)
		Premature aging (cachectic dwarfism, cataracts, lordokyphosis, loss of subcutaneous fat and reduced wound healing, short lifespan), infertility	(34)
		Increase in chemically induced colon and lung adenocarcinoma	(35)
	Truncation (human)	Mosaic variegated aneuploidy (increase in rhabdomyosarcoma, Wilms tumor and leukemia)	(36)
BubR1-Apc	BubR1+/− APC Min/+	Increase in spontaneous malignant colon adenocarcinoma	(35)
Bub3 (*Bub3/Bub3L*)	−/−	Embryonic lethal	(39)
	+/−	No increase in spontaneous tumorigenesis, an increase in chemically induced tumors	(39)
Bub3-Rae1	Bub3 +/− Rae1+/−	Prone to aneuploid, increase in chemically induced lung tumors	(40)
		Early aging and reduced lifespan	(61)
Chfr (*Chfr/Chfr*)	−/−	Viable; prone to polyploid; lymphoma, tumors in lung, liver and gastointestinal tract; increase in chemically induced skin cancer	(44)
	+/−	Increase in lymphoma, increase in chemically induced skin cancer	(44)
Rae1 (*Rae1/Rae1*)	−/−	Embryonic lethal	(39)
	+/−	Prone to aneuploid, increase in chemically induced lung cancer	(39)
Rae1-Nup98	Rae1+/− Nup98+/−	Prone to aneuploid (no tumorigenesis study)	(15)
Cenp-E (*CenpE/CenpE*)	−/−	Embryonic lethal	(48)
	+/−	Prone to aneuploid; increase in lymphoma and lung adenoma, decrease in spontaneous liver tumors	(48)
Cenp-E-p19ARF	Cenp-E +/− p19ARF−/−	Delay in chemically induced tumorigenesis	(48)
Apc (*Apc/Apc*)	−/−	Embryonic lethal	(62)
	+/− (Min/+)	Increase in colorectal cancer; increase in breast cancer in genetic background-dependent manner	(51,63,64)

Gene names are presented in common name in the cell cycle/mitosis research field, often originated from yeast. Nomenclatures in mouse and human are presented in parenthesis, as referenced from human genome organization database (www.genenames.org).
APC, adenomatous polyposis coli.

mitotic spindle assembly (46). Nup98 (Nucleopolin 98 kDa) is also a component of nuclear pore complexes (45). Several studies show that Nup98 is physically associated with transport factors and plays an essential role in nuclear trafficking (47), although the exact molecular basis by which Nup98 contributes to nuclear trafficking remains unclear.

An early study by van Deursen's group demonstrates that Rae1 cooperates with Bub3 in the regulation of chromosomal stability and suppression of tumorigenesis in mice (45). Recently, this group has shown that the Rae1–Nup98 complex functions to prevent aneuploidy formation through inhibiting premature securin degradation during early mitosis (15). Similar to mice deficient in spindle checkpoint components, mice with haploinsufficiency of both *Rae1* and *Nup98* display severe aneuploidy due to premature degradation of securin and sister chromatid segregation (15). An examination of chromosome numbers reveals that roughly 9% of $Rae1^{+/-}$ and one-third of $Rae1^{+/-}Nup98^{+/-}$ splenocytes are aneuploid, which is in contrast to the fact that no aneuploidy is observed in $Nup98^{+/-}$ and wild-type splenocytes (15). As the spindle checkpoint appears to be intact, it is proposed that APC/C is directly activated through a different mechanism (15). Indeed, this research team demonstrates that Rae1 and Nup98 interact with Cdc27 and APC6, components of APC/C (15). Moreover, mitotic, but not interphase, Rae1 and Nup98 interact with Cdh1, an activator of APC/C. Interestingly, these two proteins, regardless whether they are from interphase or mitotic cells, are not associated with Cdc20. Subsequent *in vitro* studies reveal that Rae1 and Nup98 inhibit ubiquitination of securin through APC/C activated by Cdh1 (15). These combined studies suggest that Rae1 and Nup98 play a critical part in regulating chromosomal segregation through the inhibition of securin ubiquitination mediated by APC/C^{Cdh1}.

Cenp-E Cenp-E is a kinesin family motor protein, and the protein expression is specific to mitosis. Cenp-E plays two roles in mitosis: (i) participating in making and/or maintaining chromosome to microtubule connection and (ii) serving a bifunctional role in the spindle checkpoint, which is to activate BubR1 and to turn off BubR1 when chromosome–spindle attachments are satisfied. The loss of Cenp-E disrupts spindle checkpoint function. Since anti-microtubule drugs such as taxol can inhibit interphase microtubule arrays that are prominent in neurons and result in neurotoxicity, it was proposed that drugs that target the mitotic motor might be a better substitute for targeting mitosis than anti-microtubule drugs (48). Also, the authors argue that other factors involved in spindle checkpoint signaling (e.g. Mad2 and BubR1) are also involved in non-mitotic functions (e.g. apoptosis and DNA replication checkpoint) and that Cenp-E is a better target to study the effect of aneuploidy produced through mitotic failure. To test the effect of Cenp-E inhibition *in vivo*, knockout mice of *Cenp-E* were generated. $Cenp\text{-}E^{+/-}$ MEFs are viable, but exhibit significantly higher aneuploid (48). In terms of tumorigenesis, mice with $Cenp\text{-}E^{+/-}$ background showed mixed results (48). $Cenp\text{-}E^{+/-}$ mice have elevated lymphomas and lung adenomas. However, $Cenp\text{-}E^{+/-}$ mice show a 50% reduced incidence of spontaneous liver tumors, and the tumor size is significantly smaller than control. Thus, the reduction of Cenp-E has an apparent protective effect on liver tumors. When *Cenp-E* heterozygousity is combined with the loss of the tumor suppressor $p19ARF$ ($Cenp\text{-}E^{+/-}p19ARF^{-/-}$), a majority of the animals show strong delay in tumorigenesis. Thus, Cenp-E reduction and the resulting increase in aneuploidy appears to have a dual effect, both oncogenically and as a tumor suppressor (48).

Adenomatous polyposis coli in suppression of genomic instability and tumorigenesis

Adenomatous polyposis coli (Apc) is a multifunctional tumor suppressor protein (49,50). The *Apc* gene is mutated in most colorectal

cancers and Apc dysfunction plays an essential role in regulating the onset of colon cancer. Extensive studies have shown that Apc regulates the degradation of β-catenin (49,50), a multifunctional adapter protein/transcription factor deregulated in many cancers. Apc binds to soluble β-catenin and the axin complexes that promote phosphorylation of β-catenin, thereby stimulating degradation of the β-catenin by the proteasome pathway. One of the primary functions of Apc is to negatively regulate β-catenin accumulation and translocation to the nucleus where it binds to the sequence-specific DNA-binding proteins of the Tcf/Lef family and thereby activates the transcription of Wnt target genes (50).

A vast majority of colorectal cancers display CIN characterized by loss of heterozygosity, gross chromosomal rearrangement and aneuploidy. Cell lines developed from colon cancers with the CIN phenotype have an accelerated rate of chromosome mis-segregation during cell division (51,52). Many CIN cancers appear to have acquired defects in the spindle checkpoint, which monitors the fidelity of chromosomal segregation during mitosis. Recently, it has been demonstrated that Apc may be a major player in the generation of CIN (53–55). Apc regulates the assembly of microtubules, the fibers that make up the mitotic spindle, and it physically interacts with and stabilizes the microtubules (56). Apc localizes to the plus end of microtubules that connect the mitotic spindle to the docking sites of chromosomes (kinetochores) (57). Apc directly binds to Bub1 and Bub3, two spindle checkpoint components known to reside at the kinetochores, and a truncated form of Apc results in defects in chromosomal segregation (54). These observations suggest that the truncation of Apc abolishes its interaction with microtubules, which contributes to CIN in cancer cells. Several independent studies using mouse embryonic stem cells homozygous for Min fully support this notion. Apc-mutant embryonic stem cells display extensive chromosomal and spindle abnormalities; Apc accumulates at kinetochores during mitosis and cells with mutant Apc form mitotic spindles that fail to properly attach to the kinetochores (53). In addition, a single-mutant *Apc* allele is capable of predisposing cells to increased mitotic abnormalities (58), which would contribute to tumor development. In fact, *in vitro* reconstitution experiments using cytostatic factor *Xenopus* extracts reveal that Apc protein is required for the formation of robust spindles (57).

Animal models for screening for anticancer compounds

In developing anticancer compounds, it is desirable to have a compound that kills cancer cells but spares normal cells. The aneuploidy-prone cells may provide a useful tool for searching for such compounds. As we have described above, there are several mice and cancer cells with aneuploidy-prone genetic backgrounds. Since a high incidence of aneuploidy formation is a hallmark of cancer, the aneuploidy- and cancer-prone mice models may represent an aspect of cancer cells and may be used as a useful tool for identifying anticancer compounds.

Another use of genetically defined cancer model systems is a focused approach to the signaling pathway. The SAC function is essential for mammalian cell survival, and complete loss of the SAC function kills cells through intolerably high genetic instability and/or triggering an apoptotic pathway (19,34). If aneuploid cancer cells already have a weak spindle checkpoint function, it may be possible to eradicate the cancer cells by further weakening spindle checkpoint function with a secondary drug that targets spindle checkpoint function. Some already proven anticancer drugs may at least in part eradicate cancer cells in this fashion. An Histone DeACetylase (HDAC) inhibitor, suberoylanilide hydroxamic acid, has been approved by the USA Food and Drug Administration for leukemia therapy under the name Vorinostat. Cell biological analysis indicated that HDAC inhibitor treatments weaken spindle checkpoint function and allow mitotic slippage (59,60). Whether suberoylanilide hydroxamic acid is more efficient in eradicating cancers with weaker spindle checkpoint function is an interesting issue to investigate.

As stated above, we have several genetically defined cancer model systems. They will provide tools for screening drugs, for searching signaling pathway-specific secondary drug or elucidating drug action *in vivo*.

Summary

Malignant transformation occurs when a set of genes are functionally inactivated or activated. An underlying CIN is required for the generation of multiple lesions that are characteristic of cancer. Although aneuploidy contributes to tumor progression, it remains unclear as to whether CIN is the fundamental cause for initiation of cancer. Mutations in genes involved in the regulation of key processes of chromosome segregation (e.g. SAC gene mutation) are not prevalent in primary sequence level. However, a series of mammalian genetic studies, with cultured cells and with genetically engineered mice, have demonstrated a strong link between deregulation of genes involved in regulating chromosomal segregation during mitosis and development of aneuploidy and cancer. Defects of transformed cells in cell cycle checkpoints may increase the sensitivity of these cells to additional insults to cell cycle machineries, therefore rendering them more susceptible than normal cells to chemotherapeutic agents. Mice deficient in various mitotic checkpoint components such as those summarized in this review can be excellent models for validating existing promising anticancer compounds and screening for new ones for perturbation of checkpoint pathways to induce apoptosis of tumor cells.

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