Mitotic checkpoint defects in human cancers and their implications to chemotherapy

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1. ABSTRACT

The mitotic checkpoint, also known as spindle assembly checkpoint, is to ensure accurate chromosome segregation by inducing mitotic arrest when errors occur in the spindle structure or in the alignment of the chromosomes on the spindle. Loss of mitotic checkpoint control is a common event in human cancer cells, which is thought to be responsible for chromosome instability frequently observed in cancer cells. Several reports have shown that cells with a defective mitotic checkpoint are more resistant to several types of anticancer drugs from microtubule disruptors to DNA damaging agents. In addition, inactivation of key mitotic checkpoint proteins such as BUB (budding uninhibited by benzimidazole) and MAD (mitotic arrest deficient) is influential in drug resistance in mitotic checkpoint defective cancer cells. The mitotic checkpoint has also been linked to DNA damage response and a defective mitotic checkpoint confers cancer cells resistance to certain DNA damaging anticancer drugs. This review presents recent evidence on mitotic checkpoint defects in human cancers and their association with resistance to anticancer drugs. In addition, the clinical importance and potential therapeutic implications of targeting the mitotic checkpoint to reverse drug resistance in cancer cells are also discussed.

2. MITOTIC CHECKPOINT

Mitotic checkpoint or spindle checkpoint is a surveillance mechanism to ensure that two daughter cells receive identical genetic materials after mitosis. Although the precise mechanism by which the mitotic checkpoint regulates the segregation of chromosomes during mitosis is not completely understood, most of the mitotic checkpoint proteins identified to date are mainly localized to the kinetochore, suggesting that mitotic checkpoint signaling is generated from the kinetochore. The kinetochore is a proteinaceous structure located at the centromere of each sister chromatid. During mitosis, each duplicated chromatid pair is attached to the kinetochore through binding to microtubules and aligned at the metaphase plate. Mitotic checkpoint is activated when the kinetochore fails to attach to microtubules, generating a 'wait anaphase' signal to prevent anaphase onset. The mitotic checkpoint is thought to be constitutively active at the beginning of mitosis when all kinetochores are unattached. The checkpoint signaling is turned off when all sister chromatids have attained bipolar attachment from two opposing centrosomes during metaphase. A defective mitotic checkpoint may lead to uneven chromosome segregation in two daughter cells (1-3). It has been shown that even a single unattached kinetochore is sufficient to activate the mitotic checkpoint

to prevent the onset of anaphase (4), suggesting high sensitivity and efficiency of the mitotic checkpoint in detecting chromosomal misalignment. Chromosome missegregation can lead to chromosomal instability and tumorigenesis.

3. KEY REGULATORS OF MITOTIC CHECKPOINT

Onset of anaphase and segregation of sister chromatids are initiated by a multi-subunit ubiquitin ligase, anaphase-promoting complex/cyclosome (APC/C). This ligase controls the timely degradation of cell cycle kinases and mitotic regulators which are essential for cell cycle progression. Substrate specificity is facilitated by its two co-factors, CDC20 and CDH1. During early mitosis, APC/C is activated after binding to CDC20 which recruits substrates for APC/C-dependent polyubiquitination. The ubiquitinated substrates are then subjected to 26S proteasome-mediated degradation. During late mitosis, the binding of CDH1 to APC/C leads to its activation. The subcellular localization and function of CDC20 and CDH1 are tightly regulated through phosphorylation, ubiquitination and protein-protein interaction (5-7). Degradation of two substrates, securin and cyclin B, is essential for the initiation of anaphase. Securin interacts with a caspase-related protease (named separase) to inhibit its proteolytic action on one of the subunits of cohesin (Scc1) which is responsible for holding sister chromatids together. In addition, separase is also inhibited by the cyclin-dependent kinase 1 (CDK1)/cyclin B through phosphorylation. Therefore, the APC/C-mediated destruction of securin and cyclin B results in the release and activation of separase, which in turn mediates cleavage of cohesin, leading to the initiation of segregation of sister chromatids. On the other hand, degradation of cyclin B results in CDK1 inactivation leading to mitotic exit (8,9).

The "stop anaphase" signal generated from kinetochores is suggested to consist of complexes of several mitotic regulators which bind to and inhibit APC^{CDC20}. Mitotic checkpoint proteins were firstly identified in budding yeast through genetic screening including mitotic arrest deficient (MAD) 1-3 and budding uninhibited by benzimidazole (BUB) 1-3, and the monopolar spindle 1 (MPS1) kinase (10). Vertebrate homologues of MAD1, MAD2, BUBR1, BUB1, BUB3 and MPS1 have also been identified (11-14). In addition to these core components of the mitotic checkpoint, centromeric protein C (CENP-C) (15), centromeric protein E (CENP-E) (16), ZW10-ROD-Zwilch protein complex (17,18), TAO1 kinase (19), and several other proteins (2) have also been shown to be required for mitotic checkpoint control.

4. MITOTIC CHECKPOINT SIGNALING

The presence of unattached kinetochores recruits the components of the mitotic checkpoint to generate diffusible inhibitors of CDC20 to inactivate APC/C. Although the molecular mechanisms leading to the generation of anaphase inhibitors have not been fully elucidated, it is demonstrated that many mitotic checkpoint

proteins such as MAD1, MAD2, MPS1, BUB1, BUB3, BUBR1 and CENP-E proteins localize to unattached kinetochores (20), indicating the importance of kinetochore in the regulation of mitosis. In the absence of microtubule attachment to the kinetochore, the motor protein CENP-E is not bound by microtubules and is able to activate the kinase activity of BUBR1 (16,21). The kinase activity of MPS1 is also activated which is required for the recruitment of CENP-E and MAD1-MAD2 complex to the unattached kinetochore (14). Moreover, BUB3 is responsible for localization of BUB1 and BUBR1 to unattached kinetochores (13). The co-operative interaction and synergistic action between these components are essential for the efficient generation of diffusible 'stop anaphase' signals. The signals are thought to consist of multiple complexes of MAD2-CDC20, BUBR1-BUB3-CDC20 (22) and MAD2-BUBR1-BUB3-CDC20 (23). The formation of these complexes inhibits the APC/C activator, CDC20, leading to inactivation of APC/C. While the mechanisms for inhibition of APC/C are obscure, the inhibitory complexes have recently been shown to remain associated with the APC/C (24). It is also proposed that the binding of MAD2 to MAD1 on unattached kinetochores may form a template for activating additional MAD2 molecules into active conformation capable of inhibiting CDC20 (25, 26) (Figure 1).

Generation of anaphase inhibitors is silenced only when the kinetochore on each pair of sister chromatids attaches to microtubules emanating from two spindle poles in a bipolar orientation, which then triggers the release of MAD2 from the mitotic inhibitory complex. This process may involve the suppression of BUBR1 activity when CENP-E or other proteins sense the capture of microtubules onto the kinetochores (21). A new MAD2 binding protein, CMT2/p31^{comet}, has been shown to compete with the active form of MAD2 causing the inhibition of the mitotic checkpoint (27-29). Mechanistically, the shut-off of the checkpoint is mediated through APC/C-dependent ubiquitination of CDC20 and subsequent dissociation of checkpoint proteins from the APC/C-CDC20 complex (5). As a result, the active APC/C-CDC20 becomes available to induce the onset of anaphase through destruction of securin and cyclin B. On the other hand, CDC20 is deubiquitinated by ubiquitin-specific protease 44 (USP44), another regulator of the mitotic checkpoint. While USP44 is not required for sensing unattached kinetochores, it stabilizes the inhibitory complexes such as MAD2-CDC20, thereby preventing premature activation of the APC/C (30).

5. ABERRANT MITOTIC CHECKPOINT GENE EXPRESSION AND TUMORIGENESIS

One of the hallmarks of almost all solid tumor cells is aneuploidy because majority of cancer cells contain abnormal number of chromosomes. Although it might also act to suppress tumor formation under certain circumstances (31), aneuploidy is generally thought to facilitate tumorigenesis through loss of heterozygosity of tumor suppressor genes or amplification of oncogenes (32). Conceivably, acceleration of chromosomal gains and losses, termed chromosomal instability (CIN), may cause
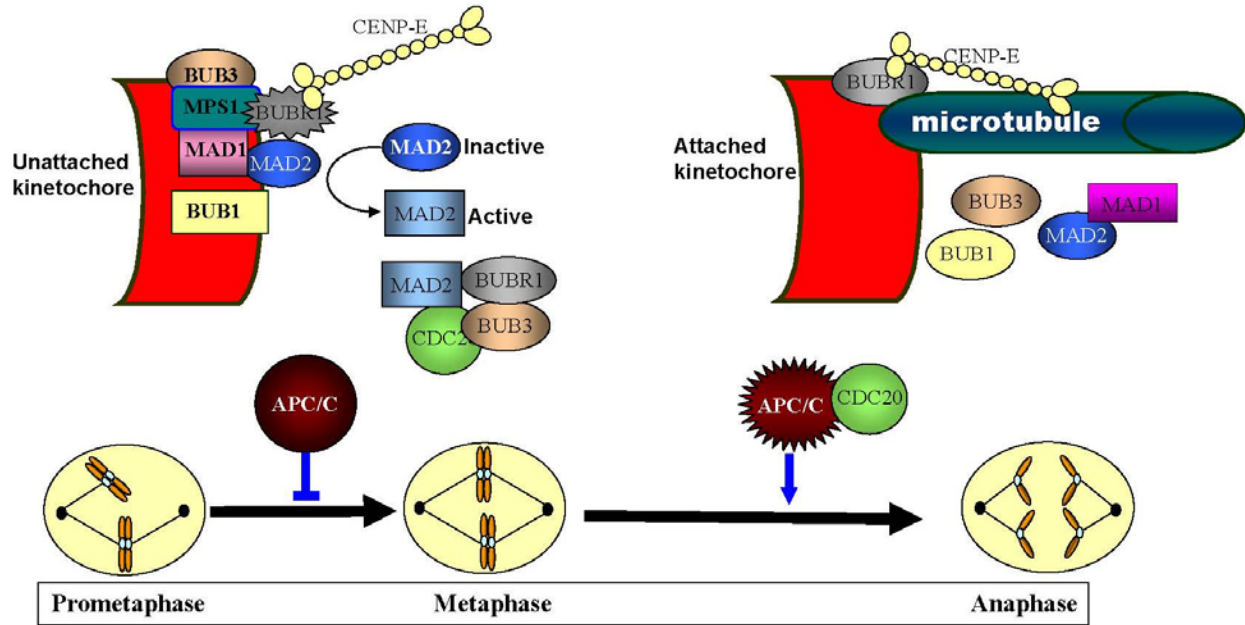
Figure 1. The mitotic checkpoint. Key regulators of the mitotic checkpoint are shown. An unattached kinetochore activates mitotic checkpoint control mechanism leading to inactivation of APC/C and cell cycle arrest. The mitotic checkpoint is turned off when all kinetochores are attached to microtubules and the binding between CDC20 and APC/C occurs.

aneuploidy and tumorigenesis. Since mitotic checkpoint is a cellular surveillance mechanism to maintain accurate segregation of sister chromatids into two daughter cells, defects in mitotic checkpoint play a key role in inducing aneuploidy (33-35).

Expression of mitotic checkpoint genes is essential for the viability of mouse and human cells. Homozygous deletion of *MAD1*, *MAD2*, *BUBR1* or *BUB3* genes in mice results in early embryonic lethality probably due to massive chromosome loss and extensive cell death (36-38) (39). In human cells, complete depletion of *MAD2* or *BUBR1* by RNA interference also leads to cell death because of massive chromosome mis-segregation after several cycles of mitosis (40,41), suggesting that a functional mitotic checkpoint is required for cell survival. Accumulating evidence suggests that an impaired but not complete loss of mitotic checkpoint response may cause aneuploidy and tumorigenesis. For example, haplo-insufficiency of MAD2 by deleting one allele of the *MAD2* gene results in cancer predisposition in mice which develop spontaneous lung tumors at an increased frequency (42). Haplo-insufficiency of other components of the mitotic checkpoint such as MAD1, BUB3 and BUBR1 in heterozygous knockout mice also exhibits genome instability with an elevated occurrence of various neoplasms including carcinogen-induced tumors (39,43,44). Studies of mouse embryonic fibroblasts extracted from these mutant mice also show that haplo-insufficiency of *MAD1, MAD2, BUB3* or *BUBR1* leads to a compromised mitotic checkpoint response and aneuploidy in response to microtubule stress (39,42-44), supporting the notion that a compromised mitotic checkpoint response contributes to aneuploidy and tumorigenesis. However,

overexpression of MAD2 in a transgenic mouse model has recently been shown to also induce tumors in multiple organs, but the high levels of MAD2 are not necessary for maintaining the malignant phenotype. In addition, this transient overexpression of MAD2 is able to promote tumorigenesis induced by the c-myc oncogene (45). Similar to the MAD2 defective mouse (42), mice overexpressing MAD2 also undergo frequent chromosomal missegregation and accumulation of aneuploid cells (45); however, these mice form much wider range of tumors which are much more aggressive compared to the mice with low MAD2. Furthermore, MAD2 is found to be a direct target of E2F and overexpressed in RB defective cancer cells with high E2F activity (46). These results suggest that while a defective mitotic checkpoint caused by reduced expression of certain mitotic checkpoint proteins may lead to a defective mitotic checkpoint, an overactive mitotic checkpoint as the result of overexpression of MAD2, especially in the cells with pre-genetic alterations such as RB inactivation, may also lead to chromosomal instability. However, the interplay of MAD2 and RB pathway in cell cycle regulation and the specificity of the regulatory effect on MAD2 or other mitotic checkpoint proteins remain to be further elucidated.

6. MITOTIC CHECKPOINT DEFECTS IN HUMAN CANCERS

The first evidence for the contribution of a compromised mitotic checkpoint to human cancer development came from a study on the *BUB1* gene, in which sporadic mutations of the *BUB1* gene were observed in 2 out of 19 colorectal cancer cell lines (47). Further experiments showed that expression of a mutant BUB1 attenuated mitotic checkpoint and caused aneuploidy in

these cell lines, indicating the importance of BUB1 in mitotic checkpoint control. In addition, a rare childhood cancer named mosaic variegated aneuploidy was associated with the germline mutations of both alleles of the *BUBR1* gene, which were detected in 5 out of 8 patients (48). However, extensive search for mutations of mitotic checkpoint genes implicates that mutational inactivation of mitotic checkpoint regulators is a rare event (49-51). For example, in 40% of lung cancer cell lines that exhibited a defective mitotic checkpoint response, no mutation of the *MAD2* and *CDC20* genes was detected (50). Sequencing analysis of *BUB1* and *BUBR1* genes also showed that only one out of 47 lung cancer cell lines had a single nucleotide substitution in one allele of the *BUB1* gene resulting in an amino acid change at codon 209 (49), indicating that mutational inactivation may not be a common mechanism responsible for *BUB1* gene inactivation. Recently, promoter hypermethylation of the *MAD2* gene was detected in several hepatocellular carcinoma cell lines in which the expression of MAD2 was suppressed (52). In addition, increased expression of breast cancer specific gene 1 (BCSG1), which is associated with the development and advancement of breast cancer (53), was correlated with reduced expression of BUBR1 in 10 breast cancer cell lines (54). Further *in vitro* studies demonstrated that overexpression of BCSG1 resulted in degradation of BUBR1 protein via the proteasome pathway which attenuated the mitotic checkpoint response (54). In addition, several viral oncoproteins have been found to directly target mitotic checkpoint proteins. The Tax oncoprotein of human T cell leukemia virus type 1 binds and inactivates MAD1 protein (11), while SV40 large T antigen suppresses the expression of BUB1 protein, leading to a compromised mitotic checkpoint response (55). Human papillomavirus E6 oncoprotein (56) and Epstein-Barr virus EBNA3 proteins (57) can also abrogate mitotic checkpoint, leading to polyploidy or resistance to mitotic checkpoint-activating drugs. Interestingly, cellular proto-oncogenes and tumor suppressor genes can also modulate the expression and activity of mitotic checkpoint genes to exert an impact on mitotic progression. For example, c-myc is able to transactivate both MAD2 and BUBR1 which may serve as a tumor suppressor response in order to mediate a mitotic arrest to compensate for the oncogenic effect of c-myc (58). While MAD2 is a target of E2F and RB (46), p53 has been shown to repress the expression of MAD1 (59). Thus, while mutational inactivation of mitotic checkpoint genes could be an uncommon cause of mitotic checkpoint abrogation, they are modulated by various cellular and viral oncoproteins or tumor suppressors.

Although the role of mitotic checkpoint in tumorigenesis is largely unknown, limited numbers of clinical studies on human cancer specimens in the literature suggest that aberrant expression of mitotic checkpoint regulators is a frequent event in human cancer specimens (see Table 1 for summary). Even though mutations of the mitotic checkpoint genes are rare events, the expression levels of corresponding proteins vary in different tumors and even within the same type of cancer. For example, while reduced BUB1 expression was found in high percentage (21/67) of colorectal cancer specimens in one

study (62), only a small percentage (3/103) of cases showed an overexpression of BUB1 (61). In addition, both overexpression and reduced expression of MAD2 have been reported in several types of cancers. Given the fact that human mitotic checkpoint genes were only identified over a decade ago and the significance of mitotic checkpoint in tumorigenesis has just begun to be understood, it is not surprising that the inconsistent results may be partly due to the lack of reliable commercial antibodies for immunoblotting and immunohistochemical studies. In contrast, results generated from human cancer cell line studies on the association between mitotic checkpoint protein expression and mitotic checkpoint control seem to be more consistent. For instance, high percentage of colon (79), lung (50,80), ovarian (81) and oral (82) cancer cell lines as well as nasopharyngeal (83) and hepatocellular (84) carcinoma cell lines fail to arrest in mitosis in response to microtubule disruption. These results suggest that defective mitotic checkpoint is common in human cancer cells. Recently, aberrant expression of certain mitotic checkpoint proteins such as MAD2 is observed in several types of cancer cell lines, including breast (12,85), lung (75), nasopharyngeal (83), ovarian (81) and hepatocellular carcinomas (52,86) (see Table 2 for summary). These results seem to be contradictory to the results from clinical specimens that increased MAD2 expression is often found in cancer specimens compared to normal tissues. It is possible that the establishment of *in vitro* cell culture may positively select cells with lower mitotic checkpoint protein expression which may provide growth advantage as demonstrated in previous mouse studies (42-44,87). Future investigations are necessary to elucidate the role of mitotic checkpoint in *in vivo* and *in vitro* survival of cancer cells.

7. ASSOCIATION BETWEEN MITOTIC CHECKPOINT DEFECTS AND SENSITIVITY TO CHEMOTHERAPEUTIC DRUGS

As discussed above, complete loss of mitotic checkpoint control leads to cell death, possibly due to severe loss of genetic materials essential for survival as the result of massive chromosome missegregation (40,41). A weakened mitotic checkpoint, however, provides survival advantage and promotes tumorigenesis, which is associated with increased aneuploidy (42-44,87). In human cancer cells, a defective mitotic checkpoint commonly confers a growth advantage enabling cells to tolerate aneuploidy and to escape from apoptosis (95,96).

7.1. Taxanes
Although microtubule disrupting chemotherapeutic drugs such as taxanes (i.e. Paclitaxel, Docetaxel) and vinca alkaloids (i.e. vincristine, colchocine) are widely used for the treatment of human cancer, surprisingly, the roles of mitotic checkpoint in relation to these types of antimitotic drugs have not been well studied (97). The taxanes enhance tubulin polymerization by inhibiting microtubule disassembly, which then prevents the breakdown of microtubules that is also required for cell cycle progression (98). In contrast, the vinca alkaloids bind to tubulin subunits and inhibits microtubule

Mitotic checkpoint defects in human cancers

Table 1. Attenuated expression of key mitotic checkpoint genes/proteins in human cancer specimens

Responsible mitotic genes	Protein Function in mitotic checkpoint	Tumour type	Positive/total No. of cancer samples	Attenuated protein /gene expression	Gene alteration	References
BUB1	Inhibits CDC20 by phosphorylation; Required for recruitment of BUBR1, MAD2 and CENP-E to kinetochores	Breast cancer	208/270	Overexpression		60
		Colorectal cancer	3/103	overexpression		61
			21/67	Reduced expression		62
		Gastric cancer	36/43	Overexpression		63
			80	No difference		64
			22/49	mutation		65
		Lung cancer	3/109	Reducedexpression		66
			1/30		Mutation	67
			1/88		mutation	49
		Melanoma	21/30	overexpression		68
		Renal cell carcinoma	Unspecified/30	No difference		69
		Salivary gland tumors	21	overexpression		70
		Thyroid cancer	1/19		mutation	71
BUBR1 (BUB1b)	Inhibits APC/C activity by direct binding	Bladder cancer	35/104	overexpression		72
		Breast cancer	208/270	Overexpression		60
		Colorectal cancer	3/109		Mutation	61
		Gastric cancer	28/43	Overexpression		63
		Renal cell carcinoma	Unspecified (n=30)	No difference		69
		Thyroid cancer	1/19		Mutation	71
BUB3	Part of APC/C inhibitory complex	Gastric cancer	34/43	Overexpression		63,65
MAD1	Recruits MAD2 to unattached kinetochores	Breast cancer	22/66	Reduced expression		73
		Gastric cancer	7/14	Reduced expression		74
		Renal cell carcinoma	Unspecified (n=30)	Reduced expression		69
MAD2	Part of APC/C inhibitory complex, inhibits APC/C activity	Bladder cancer	Unspecified (n=95)	Overexpression		46
		Breast cancer	1/48		Mutation	75
		Gastric cancer	23/54		Mutation	65
			Unspecified (n=32)	Overexpression		76
		hepatocarcinoma	Unspecified (n=82)	Overexpression (cDNA array)		77
		Lung cancer	0/30	Mutation		75
		lymphoma	107/281	Overexpression		45
		Neuroblastic tumours	Unspecified (n=106)	Overexpression (cDNA array)		46
		Renal cell carcinoma	Unspecified	Reduced expression		69
		Testicular germ cell tumour	Unspecified	Downregulation Mislocalization		78

Note : Unless indicated, expression level refers to protein level.

Table 2. Defective mitotic checkpoint control in human cancer cell lines

Tumour type	% of mitotic checkpoint defect	No of cell lines examined	Aberrant mitotic protein expression	References
Breast	78	7/9	ND	88
	100	1/1	ND	12
	70	7/10	ND	89
Colorectal	100	3/3	BUB1 mutation (2/3)	47
Head and neck	100	6/6	ND	90
Hepatocarcinoma	55	6/11	Reduced MAD2 (6/11)	86
lung	44	4/9	No MAD2 mutation	91
	50	1/2	No difference in MAD1 or MAD2 expression	80
Nasopharyngeal	40	2/5	Reduced MAD2 expresion ?(2/5)	83
ovarian	43	3/7	Reduced MAD2	81
pancreatic	100	1/1	BUB1 mutation	92
T-cell leukemia	100	6/6	mislocalization of MAD1 and MAD2(6/6)	93
Testicular germ cell tumour	75	6/8	Reduced MAD2 (5/8), MAD2 mislocalization (1/8)	94
Thyroid cancer	62.5	5/8	BUBR1, BUB1 mutation(1/8)	71

ND: Not determined.

polymerization, thus disrupting spindle dynamics and inducing a mitotic block (99). Therefore, vincristine treatment may lead to unattached kinetochores, while taxol treatment may have little effect on the kinetochore alignment of chromosomes. Since the mitotic checkpoint senses microtubule attachment to kinetochore, the function of mitotic checkpoint may be more important in modulating the effect of vinca alkaloids than taxanes. Indeed, it was reported that reduced expression of MAD2 in nasopharyngeal carcinoma cells was correlated with decreased sensitivity to taxol but not vincristine. Overexpression of MAD2, on the other hand, led to

Table 3. Summary of association between mitotic checkpoint protein expression and sensitivity to microtubule disrupting anticancer drugs in human cancer cells

Mitotic protein	Misregulation	Anticancer drug	Cancer type	Phenotype	Mechanism	References
BUB1	ND	ND	ND	ND	ND	ND
BUBR1	Suppression	Taxol	Breast cancer	Resistance	Mitosis dependent	100,102
	Mutation	Taxol	Breast cancer		Mitosis dependent	65,102
	suppression	Taxol	ovarian	Acquired resistance	Mitosis depednent	103
BUB3	Inactivation	Taxol	Cervical	Resistance	Abolishment of mitosis	104
MAD1	Suppression	Taxol	Colon cancer	No-change	Mitosis dependent	101
MAD2	Suppression	Taxol	Breast cancer	Resistance	Mitosis dependent	102
	Suppression	Taxol	Colon cancer	Resistance	Resistant to apoptosis	101
	Overexpression	Vincristine	Nasopharyngeal Carcinoma	Sensitivity	Raf/Bcl-2 phosphorylation	95
	Suppression	Taxol, Vincristine	Gastric cancer	Resistance	Bcl-2 upregulation	78
	Low expression	vincristine	Pediatric solid tumours (n=26)	Poor prognosis	mitosis dependent	105

ND: Not determined.

Table 4. Summary of aberrant mitotic checkpoint protein expression and sensitivity to DNA damaging anticancer drugs in human cancer cells

Mitotic protein	Mis-regulation	Anticancer drug	Cancer type	Phenotype	Mechanism	References
BUB1	ND	ND	ND	ND	ND	ND
BUBR1	Suppression	Aphidicolin	Colon cancer	Resistance	ND	117
BUB3	ND	ND	ND	ND	ND	ND
MAD1	ND	ND	ND	ND	ND	ND
MAD2	High expression levels	Cisplatin	Testicular germ cell tumour	Sensitivity	MEK/ERK activation; Activation of apoptosis	78
	Overexpression	Cisplatin; γ-irradiation	Nasopharyngeal carcinoma	Resistance	Bcl-2 upregulation	96
	Suppression	Adiamycin; Cisplatin	Gastric cancer	Resistance	Mitotic defect	78
	Gene deletion	Topo II inhibitor	Colon cancer	Resistance	Mitotic defect	116
	Suppression	Aphidicolin	Colon cancer	Resistance	Mitotic defect	117
	Supression	Adriamycin, Chk1 inhibitor (UCN-01)	Cervical carcinoma	resistance	Resistant to Mitosis dependent apoptosis	118
Msp1	Suppression	SN-38	Colon cancer	Resistance	Mitotic activation and apoptosis	119

ND: Not determined.

promotion of taxol-induced but not vincristine-induced apoptosis (95). As expected, the MAD2-mediated taxol sensitivity was associated with a prolonged mitotic arrest which results in subsequent activation of apoptosis pathway (95). Another study on breast cancer cells showed that suppression of *MAD2* and *BUBR1* expression respectively by RNAi led to abolishment of mitotic checkpoint function and resistance to paclitaxel (100). However, overexpression of MAD2 in the *BUBR1* knockout breast cancer cells failed to enhance the sensitivity to taxol (100), suggesting that BUBR1 is indispensable for a competent mitotic checkpoint. These results are further supported by a recent study on human breast and cervical cancer cell lines, in which the presence of BUBR1 was essential for taxol-induced mitotic arrest and subsequent apoptosis (100). On the other hand, partial downregulation of MAD1, a binding partner of MAD2 protein during mitosis, did not confer resistance to taxol in human colon cancer cell lines, although an impaired mitotic checkpoint and aneuploidy were observed (101). Thus, a functional mitotic checkpoint may be necessary for microtubule disrupting anti-cancer drug-induced cell death by promoting a mitotic arrest and subsequent apoptosis. Table 3 summaries the association between dysregulaton of

mitotic checkpoint regulators and sensitivity of human cancer cell lines to microtubule disrupting anticancer drugs.

7.2. DNA damaging agents

Recently, it has been suggested that DNA damage is able to activate the MAD2-mediated mitotic checkpoint which enhances cell survival and genome stability in yeast (106). On the other hand, the activation of mitotic checkpoint induces phosphorylation changes in DNA checkpoint proteins Rad53 and Rad9 (107). In addition, one of the key factors in DNA damage response, Chk1 (checkpoint kinase 1), has been reported to play a key role for mitotic progression. Depletion of Chk1 not only causes premature mitotic entry but also leads to chromosome misalignment, kinetochore defects, and increased resistance to taxol (108,109). Chk1 is also required for phosphorylation of Aurora-B kinase and BUBR1 (109). These and other related findings suggest a cross-talk between the mitotic checkpoint and DNA damage checkpoint (110). In support of this model, genetic studies in yeast showed that disruption of yeast *MAD2* gene was able to suppress mitotic arrest induced by a DNA replication inhibitor, hydroxyurea, and a DNA damaging agent, methyl methanesulfonate (111). Disruption of genes

involved in DNA replication also resulted in replication block and mitotic arrest which could be partially relieved by simultaneous deletion of the yeast *MAD2* gene (111). In other words, MAD2-mediated mitotic arrest plays a role in responding to DNA damaging agents in yeast cells. A compromised DNA damage response was also observed in the *BUBR1*+/- murine fibroblasts treated with doxorubicin and ultraviolet (UV) (112). Upon DNA damage, while the *BUBR1*+/+ cells showed a mitotic arrest, the *BUBR1*+/- cells continued to divide with low levels of γH2AX, p53 and p21 (112). The fact that the BUBR1 knockdown experiments in HeLa cells showed an impaired expression of both p53 and p21 further suggests a positive involvement of BUBR1 in p53-mediated DNA damage response (112). Furthermore, both BUBR1 and BUB3 have been reported to physically interact with Poly(ADP-ribose) polymerase 1(PARP-1), one of the first known members of the PARP family which plays a role in DNA damage surveillance. This physical interaction is found to mediate DNA damage response induced by γ-irradiation and doxorubicin (112,113). Since PARP-1 knockout mice show a decreased ability to repair DNA damage (114), these results further implicate a possible involvement of the spindle checkpoint in DNA damage response.

In human cancers, only recently the association between mitotic checkpoint and cellular response to DNA damaging anticancer drugs has been reported (Table 4). For example, it has been demonstrated that a prolonged period of metaphase can be observed in several human cell lines after treatment with high doses of topoisomerase II inhibitors or radiation, which induce extensive DNA double strand breaks. Such prolonged mitotic arrest appears to be MAD2-dependent because increased localization of MAD2 protein in the kinetochores was detected (115). Moreover, using live cell imaging, microinjection of a dominant-negative form of MAD2 (MAD2ΔC, which lacks the C-terminal region for CDC20 binding), inhibited the DNA damage-induced mitotic arrest and induced a rapid onset of anaphase (115). These findings suggest that MAD2-mediated mitotic checkpoint may also regulate the response to DNA damage. In addition, colon cancer cells carrying heterozygous deletion of the *MAD2* gene are resistant to topoisomerase II poisons compared to isogenic wild-type cells (116), indicating that MAD2 may play a critical role in the induction of cell death in response to DNA damaging agents. Exposure to aphidicolin and irradiation also leads to a prolonged mitotic arrest in *p53*-deficient colon cancer cells, which subsequently undergo cell death (117), suggesting that the DNA damage-induced mitotic arrest and subsequent death may be independent of p53 pathway. Partial suppression of *MAD2* or *BUBR1* expression by RNA interference is able to inhibit DNA damage-induced mitotic arrest and reduce the extent of cell death (117). These findings implicate that MAD2 and BUBR1 may be key factors in mediating cell death following mitotic arrest in response to certain DNA damaging agents. Recently, our laboratory has found that reduced expression of MAD2 correlates with cellular resistance to DNA damaging anticancer agents, such as cisplatin and γ-irradiation in nasopharyngeal carcinoma cell lines. In addition, ectopic MAD2 expression promotes chemosensitivity to cisplatin,

which is associated with a delayed mitotic arrest and increased apoptotic cell death (96). These results were confirmed recently in our study on gastric cancer cells, in which inactivation of *MAD2* through RNAi resulted in suppression of cisplatin-induced apoptosis associated with increased Bcl-2 expression (78). It is possible that in response to DNA damage, cells with sufficient MAD2 expression may be able to activate apoptosis pathway, plausibly as the result of a prolonged MAD2-mediated mitotic arrest. We found in testicular germ cell tumor cells that inactivation of MAD2 led to suppression of MEK/ERK pathway and subsequent resistance to cisplatin-induced apoptosis (78). Similar effect was also reported in mouse embryonic fibroblasts with a deleted *BUBR1* gene. DNA damage response in the *BUBR1* depleted cells was severely impaired as demonstrated by compromised induction of p53 and p21 after exposure to UV light and doxorubicin, and this process was associated with failed mitotic arrest (112). Given that BUBR1 and MAD2 are the two most well established mitotic checkpoint regulators, these results implicate mitotic checkpoint in cellular response to DNA damaging agents.

Recently, it was reported that a MAD2 homologue, MAD2B (human homologue of yeast Rev7), which shares 53% similarity in amino acid sequence to MAD2 (120), played a key role in modifying cisplatin sensitivity in human cancer cells (121). MAD2B is important in translesion DNA synthesis whose function is to overcome DNA adduct-induced replication block and loss of which leads to resistance to cisplatin (122). On the other hand, MAD2B physically interacts with MAD2 (120) and associates with CDH1 to inhibit the activity of APC/C (123,124). In light of these findings, further investigations are warranted to elucidate the exact roles of MAD2B-MAD2 complex in mitotic checkpoint control and DNA damage response.

8. CLINICAL IMPLICATIONS

Impairment of mitotic checkpoint is a common phenotype in human cancer cells. Weakened mitotic checkpoint provides an advantage for cell survival. This raises the opportunity of manipulating the mitotic checkpoint to inhibit tumor cell growth. Recent findings on the association between mitotic checkpoint and DNA damage response extend the significance of this checkpoint from microtubule disrupting agents to DNA damaging anticancer drugs. Several potential clinical implications can be derived. First, since expression of mitotic checkpoint proteins is often reduced or altered in cancer cells, restoration of their expression in cancer cells may re-establish mitotic checkpoint control leading to increased susceptibility to chemodrug-induced apoptosis. Second, while a weakened mitotic checkpoint may increase the susceptibility to tumorigenesis, complete silencing of the mitotic checkpoint may result in lethality. Promising results have recently been obtained with small molecule inhibitors that target a mitotic specific protein, KSP, which is required for spindle-pole separation. Because of their specificity on mitotic cells, these inhibitors may reduce the side effects of microtubule disruptors (125). An MPS1

inhibitor has also been obtained by screening small drug compounds (126), indicating the feasibility of these approaches. Third, a growing body of evidence suggests the importance of a functional mitotic checkpoint in regulating cell death induced by microtubule disrupting anticancer drugs such as taxol and vincristine, identification of attenuated mitotic checkpoint protein expression in human cancer specimens may be able to provide guidance for identification of patients potentially resistant to this type of anticancer drugs. Alternative anticancer drugs with different mechanisms may be considered to maximize the benefit of chemotherapy. Fourth, recent demonstration of the involvement of mitotic checkpoint in DNA damage response has led to increased research interest in its role in cellular sensitivity to DNA damaging anticancer drugs. Since DNA damaging anticancer drugs are used widely in the treatment of human cancer, identification of tumors potentially resistant to this type of chemotherapeutic drugs will greatly benefit clinical management of cancer patients. Furthermore, designing new drugs that can mimic mitotic checkpoint protein function may be able to induce chemosensitization to anticancer drugs that target mitosis and DNA, thereby reversing the drug resistance phenotype commonly observed in cancer patients. However, with limited information on mitotic checkpoint protein expression in clinical specimens, the significance of mitotic checkpoint in human cancer needs to be better defined. Clinical studies on the association between mitotic checkpoint protein expression and clinical response as well as patient survival will provide valuable information to demonstrate the importance of mitotic checkpoint in chemodrug sensitivity.

9. CONCLUSION

Defective mitotic checkpoint is a main cause of aneuploidy. Impairment of mitotic checkpoint in cells causes chromosomal instability and likely provides a growth advantage enabling the aneuploid cells to escape apoptosis and to effect uncontrolled proliferation. The fact that mitotic signaling is often defective in human cancer cells provides a target for the development of anticancer drugs. With increasing reports on attenuated mitotic checkpoint protein expression and its association with sensitivity to certain types of anticancer drugs, it is apparent that manipulation of mitotic gene expression may be a strategy to reverse drug resistance. In addition, with demonstrated specificity to microtubule disrupting agents and DNA damaging anticancer drugs, the mitotic checkpoint defective cells may be given alternative treatments which can induce cell death through mitotic checkpoint independent pathways. However, because the majority of experimental evidence was obtained from cancer cell lines, the association between aberrant mitotic checkpoint protein expression and human cancer needs to be established before its full potentials in clinical applications are explored. In addition, studies on differential mitotic checkpoint protein expression among cancer patients who show differential responsiveness to chemotherapy will be able to confirm the significance of mitotic checkpoint to chemosensitivity in patients. However, important challenges still remain as to how the wait signal is produced at the unattached kinetochores, why mitotic checkpoint defective cells can escape the apoptosis pathway, and how mitotic checkpoint triggers cell death in response to anticancer drug treatment in cancer cells. Since DNA damaging anticancer drugs are commonly used in the treatment of a majority of human cancers, further exploration of the link between mitotic checkpoint and DNA damage response pathways such as p53, ATM/ATR, will no doubt provide new insights to the importance of mitotic checkpoint in DNA damaged-induced apoptosis. Understanding the molecular mechanisms of this checkpoint and its role in cancer cell survival will provide new approaches to therapeutic interventions of human cancers.

10. ACKNOWLEDGEMENTS

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