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Mitotic Drug Targets

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ABSTRACT

Mitosis is the key event of the cell cycle during which the sister chromatids are segregated onto two daughter cells. It is well established that abrogation of the normal mitotic progression is a highly efficient concept for anti-cancer treatment. In fact, various drugs that target microtubules and thus interfere with the function of the mitotic spindle are in clinical use for the treatment of various human malignancies for many years. However, since microtubule inhibitors not only target proliferating cells severe side effects limit their use. Therefore, the identification of novel mitotic drug targets other than microtubules have gained recently much attention. This review will summarize the latest developments on the identification and clinical evaluation of novel mitotic drug targets and will introduce novel concepts for chemotherapy that are based on recent progress in our understanding how mitotic progression is regulated and how anti-mitotic drugs induce tumor cell death. J. Cell. Biochem. 111: 258–265, 2010.

KEY WORDS: MITOSIS; MICROTUBULE; TAXOL; SPINDLE CHECKPOINT; AURORA KINASE; POLO-LIKE KINASE; KINESIN; CHROMOSOMAL INSTABILITY; CENTROSOME

The progression of mitosis is a highly complex order of events. In prophase, chromosomes condense and the nuclear envelope is broken down. The duplicated centrosomes move to the opposite poles of the cell in prometaphase and a bipolar mitotic spindle is formed mainly by centrosome-mediated tubulin polymerization. Microtubules at this stage are highly dynamic structures that search for and capture chromosomes at their kinetochores by a stochastic mechanism. Stable attachment of microtubules to kinetochores is required for proper chromosome alignment in metaphase, which is mediated by the action of several mitotic kinesin motor proteins. Subsequent to complete chromosome congression on a metaphase plate, the sister chromatids separate in anaphase and are pulled towards the poles again mediated by kinesin proteins and by regulated microtubule shortening. In telophase, new nuclear envelopes are reformed in the daughter cells around the segregated chromatids and cell division is completed by cytokinesis.

THE MITOTIC SPINDLE ASSEMBLY CHECKPOINT

The key step in mitosis is the meta- to anaphase transition, where a cell must ensure that every single chromosome is properly aligned before anaphase is initiated. This crucial step is under tight control of ubiquitin-dependent protein proteolysis, which ensures the irreversibility of this transition. It requires a large ubiquitin ligase complex known as the anaphase promoting complex or cyclosome (APC/C) responsible for the ubiquitination of several key mitotic regulators including cyclin B and securin, which are subsequently degraded by the 26S proteasome. The proteolysis of cyclin B is required for mitotic exit while the destruction of securin is required for the onset of anaphase. Thus, APC/C-mediated protein proteolysis represents one of the key steps in the metaphase to anaphase transition and must be prevented in the presence of incompletely aligned chromosomes [Peters, 2006]. This level of regulation is provided by a surveillance mechanism known as the mitotic spindle assembly checkpoint (SAC), which senses the presence of unaligned chromosomes and inhibits the APC/C. The SAC involves a group of conserved checkpoint proteins including Mad1, Mad2, Bub1, BubR1, and Bub3 among others that are recruited specifically to kinetochores that are either not attached to microtubules or that do not exhibit a tension across sister kinetochores, which indicates a lack or incomplete chromosome alignment [Musacchio and Salmon, 2007]. Hence, the SAC prevents the onset of anaphase until all chromosomes have achieved full alignment by inhibiting the ubiquitin ligase activity of the APC/C.

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CHROMOSOME MAL-ATTACHMENT AND ITS CORRECTION

Proper chromosome alignment requires the bi-orientation of all chromosomes, that is, one kinetochore has to be attached to microtubules emanating from one pole while the sister kinetochore attaches to microtubules from the opposite pole (amphitelic attachments). The normal geometry of the bipolar mitotic spindle provides a bias for chromosome bi-orientation. Nonetheless, due to the randomness of microtubule–kinetochore interaction mal-attachments can naturally occur, in particular in the early phases of mitosis. These can involve attachments of one or both kinetochores to only one pole (mono- and syntelic attachments) as well as concomitant binding of one kinetochore to both poles (merotelic attachments). Normally, mal-oriented chromosomes are corrected by selective destabilization of these faulty kinetochore attachments while amphitelic attachments are further stabilized. This correction process, which is essential for correct chromosome alignment and segregation, involves the centromere-based Aurora-B kinase, which directly regulates the mitotic kinesin MCAK that functions as a microtubule depolymerase at kinetochores involved in the destabilization of microtubule–kinetochore interactions [Thompson et al., 2010].

HOW DO MICROTUBULE INHIBITORS WORK?

All currently approved drugs that target mitosis are microtubule inhibitors (see Table I for examples) that interfere with the dynamics of microtubules by directly binding to the tubulin subunits [Jordan and Wilson, 2004]. Inhibition of microtubule dynamics inevitably prevents the normal alignment of chromosomes and thus activates the SAC, which in turn mediates a mitotic arrest. Since the SAC cannot be satisfied under these conditions, the prolonged mitotic arrest is followed by the induction of apoptosis, the ultimate goal of chemotherapy (Fig. 1A). It is still unclear how the mitotic arrest is coupled to the activation of the apoptotic machinery, but a prolonged activation of the SAC seems to be an important prerequisite for the efficacy of microtubule inhibitors [Sudo et al., 2004; Kienitz et al., 2005; Gascoigne and Taylor, 2008]. Importantly, there might be significant variation in the cellular response to microtubule inhibitors. It has recently been shown that some cells die already during the SAC-mediated mitotic arrest while others, even in the same culture, exit from mitosis without executing chromosome segregation and die in the subsequent postmitotic G1 phase of the cell cycle [Gascoigne and Taylor, 2008]. Nevertheless, the treatment of cancer cells with microtubule inhibitors exerts very high efficacy in cell killing in vitro and this has also been verified in clinical applications [Jordan and Wilson, 2004]. However, taxanes, epothilones, or *Vinca* alkaloids do not exhibit a tumor cell selectivity and they affect not only proliferating cells but also target resting and differentiated cells. In fact, severe side effects accompany the therapeutic use of these drugs, largely because they interfere with microtubule function in non-tumor-derived cells. In particular, inhibition of microtubule-mediated cargo transport in axons results in pronounced neuropathies and significantly limits the use of microtubule inhibitors in patients [Jordan and Wilson, 2004; Schmidt and Bastians, 2007]. Therefore, the identification of alternative non-microtubule drug targets that circumvent these unwanted side effects and which might exhibit tumor cell selectivity are highly desired.

NOVEL MITOTIC DRUG TARGETS ALREADY INVESTIGATED IN CLINICAL TRIALS

An ideal mitotic drug target should fulfill several important criteria: (i) it should have a key role during mitosis and preferably no function in non-dividing cells; (ii) its inhibition should severely abrogate the mitotic progression leading to the induction of cell death; (iii) it should be present in cancer cells and—ideally—not present in non-transformed cells, that is, it should possess characteristics of an oncogene; (iv) it should be druggable, that is, it should possess a measurable activity that can be inhibited by small molecule drugs; and (v) it should provide robust diagnostic markers that allow a pre-selection of patients and to follow its inhibition after drug treatment.

In recent years, several next generation mitotic drug targets have emerged that match at least some of these key criteria and small molecule inhibitors have been identified that are already

TABLE I. Summary of Mitotic Drug Targets and Examples of Corresponding Anti-Mitotic Chemotherapeutic Drugs

Molecular target	Drug/compound	Clinical status
Mitotic spindle, microtubules	Taxanes (e.g., paclitaxel, taxotere)	Approved, in clinical use
	Vinca alkaloids (e.g., vinblastine, vinorelbine)	Approved, in clinical use
	Epothilones (e.g., ixabepilone, patupilone)	Approved, phase III
Plk1	BI2536 (Boehringer Ingelheim)	Phase I/II
	GSK461364A (GlaxoSmithKline, GSK)	Phase I
	NMS-1286937 (Nerviano Medical Sciences)	Phase I
Aurora-A	MLN8054 (Millennium Pharmaceuticals)	Phase I (terminated)
	MLN8237 (Millennium Pharmaceuticals)	Phase I/II
Aurora-B	MK-0457 (Merck & Co, Vertex)	Phase I/II
	AZD1152 (AstraZeneca)	Phase I/II
	PHA-739358 (Nerviano Medical Sciences)	Phase I/II
Kinesin-5/KSP	SB-715992/ispinesib (Cytokinetics/GSK)	Phase I/II
	SB-743921 (Cytokinetics/GSK)	Phase I/II
	AZD4877 (AstraZeneca)	Phase I/II
Kinesin Cenp-E	GSK923295A (Cytokinetics/GSK)	Phase I

Only drug targets are listed, for which small molecule inhibitors are either already in clinical use or currently undergoing clinical trials.

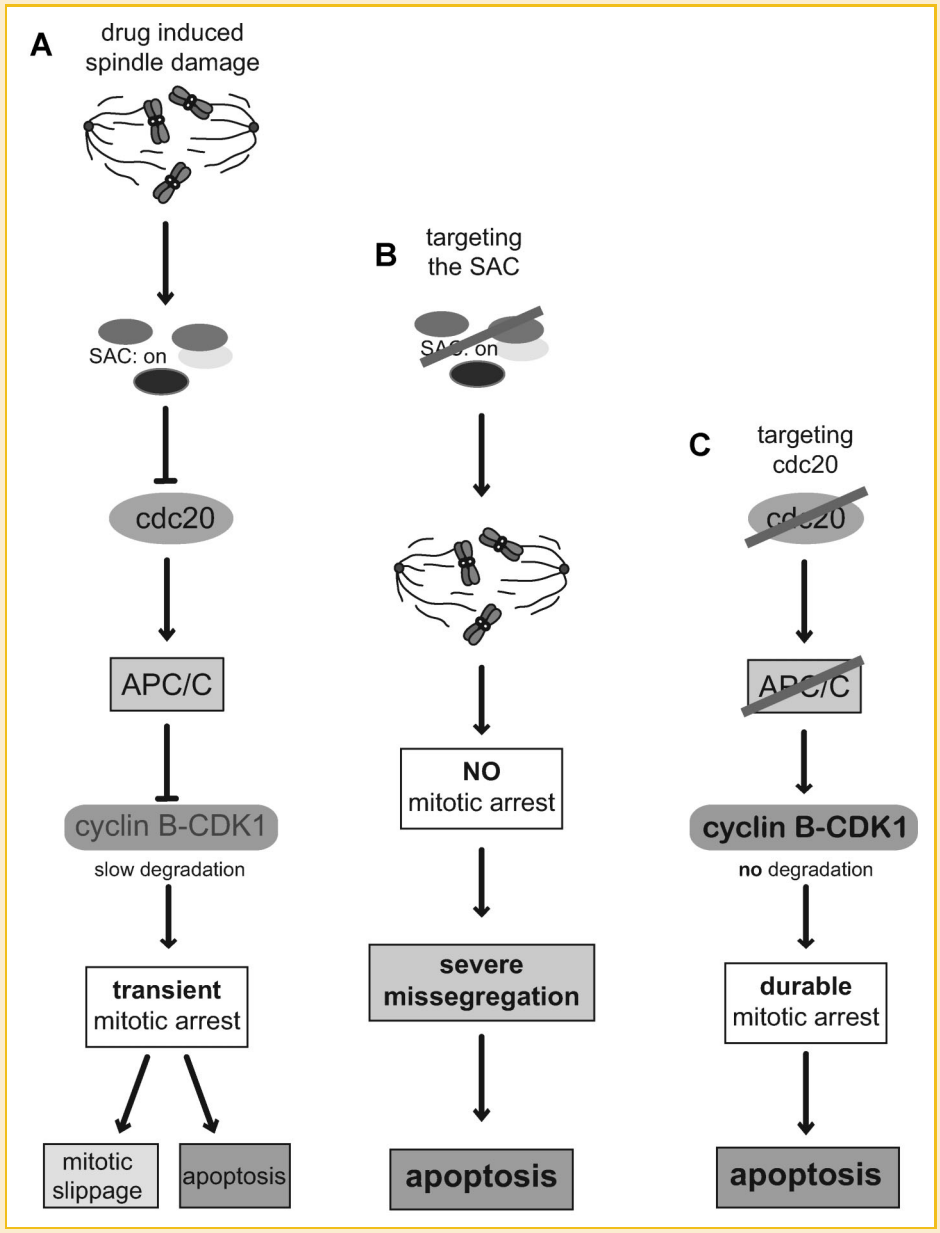

Fig. 1. The mitotic spindle assembly checkpoint and the mitotic proteolysis machinery as novel mitotic drug targets. A: Drug-induced spindle damage causes chromosome misalignment that is detected by the spindle assembly checkpoint (SAC). SAC proteins sequester Cdc20 and thus, inhibit the ubiquitin-ligase activity of the APC/C, which is responsible for the mitotic ubiquitination and degradation of cyclin B associated with a transient mitotic arrest, which can be followed by the induction of apoptosis. Due to the slow, but continuous degradation of cyclin B in the presence of an activated SAC cells can untimely exit from mitosis (mitotic slippage). B: The inhibition of the SAC allows the premature segregation of sister chromatids resulting in severe missegregation, which is no longer compatible with cell survival. C: The inhibition of Cdc20 prevents the activation of the APC/C and stabilizes cyclin B in mitosis. Consequently, cells exhibit a durable mitotic arrest, which is followed by the induction of apoptosis. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

investigated in clinical trials (Table I). These drug targets will be discussed briefly in the following sections.

POLO-LIKE KINASE 1 (Plk1)

Plk1 is a multifunctional kinase that is involved in the regulation of the G2/M transition, in the progression through, and in the exit from mitosis. According to its various functions, it localizes to centrosomes, kinetochores, and central spindle. In late G2, it is required for the maturation of centrosomes and for the initial activation of CDK1 and thus, for the timely entry into mitosis [van Vugt and Medema, 2005]. Plk1 controls various steps of mitosis including centrosome-dependent spindle assembly, regulation of kinetochore–microtubule interaction, and cytokinesis. It is also required for the activation of the APC/C by phosphorylation of several APC/C subunits and by promoting the degradation of the early mitotic inhibitor 1 (Emi1) [Barr et al., 2004; van Vugt and

Medema, 2005]. In addition, it has recently been shown that Plk1 might fulfill a role for DNA replication during S phase suggesting that Plk1 might not solely function at G2/M [Trenz et al., 2008]. From the therapeutic point of view, it is important that Plk1 is frequently overexpressed in human cancer suggesting that it might represent an oncogene. In fact, overexpression of Plk1 is sufficient to transform rodent fibroblasts and to allow xenograft tumor growth in nude mice. However, the Plk1 gene is rarely amplified in tumors and therefore, it is currently unclear whether Plk1 is indeed an oncogene in human cancer or whether its elevated expression is rather an effect of high proliferation of cancer cells [Degenhardt and Lampkin, 2010].

A growing number of small molecule inhibitors for Plk1 have been developed and several of them are currently evaluated in phase I and II clinical trials (Table I). Pre-clinical studies have demonstrated that inhibition of Plk1 is associated with severe abrogation of normal spindle assembly and chromosome alignment. Often, monopolar spindles are formed after Plk1 inhibition, which causes a prolonged activation of the SAC and thus, a mitotic arrest [Sumara et al., 2004]. Similar to microtubule inhibitors, the mitotic arrest is followed by the induction of apoptosis, but the details on how apoptosis is activated in response to Plk1 inhibition are still unclear. Interestingly, a genome-wide screen has recently uncovered a synthetic lethal interaction of *PLK1* with the *KRAS* oncogene. Accordingly, *RAS* mutant cells show a higher sensitivity for Plk1 inhibition [Luo et al., 2009], suggesting a therapeutic window for Plk1 inhibition in cancer cells with typical tumor-associated alterations. On the other hand, potent Plk1 inhibitors might also target the structurally similar Plk family members Plk2 and Plk3, which may have tumor suppressor functions. In fact, Plk3 is a positive regulator of p53 after DNA damage and *PLK2* was shown to be a transcriptional target of p53 [Degenhardt and Lampkin, 2010]. Hence, inhibition of other Plks might significantly impact on the cellular DNA damage response leading to genomic instability and bearing the risk for inducing de novo tumorigenesis.

AURORA KINASES

The Aurora-A and Aurora-B kinases are key regulators of mitosis with distinct functions that were considered as novel mitotic drug targets due to their frequent overexpression in human cancer [Keen and Taylor, 2004]. However, frequent gene amplifications in various tumor entities were only found for Aurora-A and overexpression of Aurora-A, but not of Aurora-B is sufficient to transform rodent fibroblasts suggesting that Aurora-A rather than Aurora-B might represent an oncogene [Mountzios et al., 2008].

Aurora-A is a centrosomal and spindle associated serine/threonine kinase required for mitotic entry, centrosome function, and bipolar spindle assembly. Several centrosomal and spindle proteins including TACC3 and the kinesin-5/KSP that are involved in bipolar spindle assembly are direct phosphorylation targets of Aurora-A. Accordingly, inhibition of Aurora-A by siRNAs causes highly abnormal, often monopolar mitotic spindle assembly and abrogates the normal progression of mitosis, finally leading to the induction of apoptosis, probably through elevating the rate of chromosome missegregation [Marumoto et al., 2005].

In contrast, Aurora-B is part of the so-called chromosomal passenger complex (CPC), which also includes INCENP, survivin, and borealin. The CPC has multiple functions during mitosis and is required for the mitotic phosphorylation of histone H3, for proper chromosome biorientation and alignment and for cytokinesis. Ablation of CPC function or inhibition of Aurora-B kinase activity results in severe chromosome misalignment, which is due to the inability to correct chromosome mal-attachments. In addition, in the absence of the CPC cytokinesis cannot be executed and tetraploid cells exit from mitosis without chromosome segregation [Ruchaud et al., 2007].

Intensive efforts have been made to identify small molecule inhibitors for the Aurora kinases. In fact, for Aurora-A two specific inhibitors have been described so far. MLN8054 and MLN8237 inhibit Aurora-A selectively over Aurora-B and reproduce the mitotic defects compatible with Aurora-A inhibition [Manfredi et al., 2007; Kaestner et al., 2009; Gorgun et al., 2010]. For MLN8237, several phase I and II clinical trials are currently ongoing (Table I). Most small molecules disclosed to date targeting Aurora kinases inhibit Aurora-B over Aurora-A (see Table I for a few examples). The major phenotype of drug-induced Aurora-B inhibition is the abrogation of chromosome alignment and the inhibition of cytokinesis resulting in severe polyploidization. The latter most likely contributes to the cell death inducing activity of Aurora-B inhibitors. Several Aurora-B inhibitors are currently investigated in various clinical trials [reviewed in Keen and Taylor, 2004; Schmidt and Bastians, 2007; Mountzios et al., 2008].

THE MITOTIC KINESIN-5 (KSP, Eg5, Kiff11)

Mitotic kinesins are motor proteins that play important roles in centrosome positioning, chromosome congression, and segregation [Miki et al., 2005]. The interest in mitotic kinesins as novel drug targets was initiated by the identification of monastrol as the first small molecule inhibitor of the kinesin-5, also known as kinesin spindle protein (KSP), Eg5, or Kif11 [Mayer et al., 1999]. Kinesin-5 is required for the movement of the two centrosomes to the cell poles and hence, its inhibition by monastrol results in the formation of monastrol, but fully dynamic mitotic spindles. This leads to mono-oriented chromosome attachments that are recognized by the SAC. Similar to a treatment with microtubule inhibitors, a prolonged SAC-mediated mitotic arrest is followed by the induction of apoptosis by a largely unknown mechanism [Huszar et al., 2009]. The unique role of kinesin-5 during mitosis makes this kinesin an important mitotic drug target. Accordingly, since the discovery of monastrol many kinesin-5 inhibitors have been identified and anti-tumor activity has been demonstrated. Currently, several of these inhibitors are evaluated in clinical trials (see Table I for examples) and first results verified that typical microtubule inhibitor associated side effects are not observed for kinesin-5 inhibitors [Huszar et al., 2009].

THE MITOTIC KINESIN Cenp-E

Given the great success of kinesin-5 as a mitotic drug target, other mitotic kinesins might exhibit high potential as drug targets as well. One of these promising candidates is centromeric protein E (CENP-E). Cenp-E is a kinetochore-based motor protein required for chromosome alignment and perhaps for regulating BubR1, a protein kinase involved in SAC signaling and chromosome alignment [Wood et al., 1997; Mao et al., 2003]. Importantly, no role has been found for Cenp-E outside of mitosis. Most recently, the first inhibitors for Cenp-E were disclosed (Table I) and it has been shown that inhibition of Cenp-E either by siRNA or by small molecule inhibitors results in a mitotic delay associated with misaligned chromosomes followed by the induction of apoptosis [Henderson et al., 2009; Wood et al., 2010]. Importantly, anti-tumor activity has been demonstrated for one Cenp-E inhibitor and the first clinical trials are already underway [Wood et al., 2010]. However, it is important to note that partial inhibition of Cenp-E might bear the risk to induce chromosome missegregation and aneuploidy, which might in turn contribute to tumor growth.

NEW THERAPEUTIC CONCEPTS TARGETING MITOSIS

In addition to the mitotic targets that are already evaluated in clinical trials, several novel strategies targeting mitosis have been suggested. These conceptual ideas are based on most recent work examining the mechanisms of chromosomal instability associated with mitotic abnormalities that are typical for human cancer. In particular, the mitotic spindle checkpoint and the generation of correct amphitelic microtubule–kinetochore attachments seem to play key roles in the maintenance of chromosomal stability and could represent promising points for therapeutic intervention.

TARGETING THE MITOTIC SPINDLE ASSEMBLY CHECKPOINT

The mitotic SAC is a key mitotic surveillance pathway that guards against chromosome missegregation [Musacchio and Salmon, 2007]. In fact, lowering the expression of various SAC genes in human tissue culture cells by siRNAs or in heterozygous mouse knockout models indicated that a compromised SAC causes chromosome missegregation leading to aneuploidy and associated with tumorigenesis [e.g., Taylor and McKeon, 1997; Kalitsis et al., 2000; Michel et al., 2001; Kienitz et al., 2005]. In line with this, mutations or altered SAC gene expression has been found in aneuploid human cancer cells, albeit at low frequency [Cahill et al., 1998; Wang et al., 2002]. In contrast, complete abrogation of SAC function in homozygous SAC knockout mice results in embryonic lethality suggesting that the SAC itself is essential for cell viability [Dobles et al., 2000; Foijer et al., 2008]. Accordingly, it has been shown that the significant repression of *MAD2* or *BUBR1* gene expression in human cancer cell lines induces massive chromosome missegregation, which led to apoptosis within a few cell cycles indicating that severe chromosome missegregation induced by SAC abrogation is no longer compatible with cell survival [Kops et al., 2004; Michel et al., 2004]. Thus, in sharp contrast to other cell-cycle checkpoint pathways the SAC represents a checkpoint that is required for cell survival and this finding has led to the idea that inhibition of the SAC might represent a novel therapeutic strategy of anti-cancer treatment (Fig. 1B). Following this idea, small molecule inhibitors that override a SAC-mediated mitotic arrest in the presence of unaligned chromosomes have been identified [DeMoe et al., 2009; Stolz et al., 2009; Kwiatkowski et al., 2010]. Among them, inhibitors of the Aurora kinases as well as of the checkpoint kinase Mps1 showed high potential to inhibit the SAC leading to severely elevated levels of chromosome missegregation. Importantly, some of the inhibitors exhibit a strong apoptosis inducing activity in human cancer cell lines while non-transformed cells showed reduced sensitivity towards SAC inhibition [Stolz et al., 2009; Kwiatkowski et al., 2010]. Although the basis for this potential tumor cell selectivity is unclear it might provide a therapeutic window for which SAC inhibitors can be used as anti-cancer drugs. However, in vivo studies examining tumor growth after inhibition of the SAC are needed to verify these initial results.

INHIBITION OF THE SAC AND SENSITIZING CANCER CELLS TO MICROTUBULE INHIBITORS

In contrast to the inhibition of the SAC as a potential stand-alone therapy, it has recently been suggested to combine a partial SAC inhibition with sub-lethal doses of microtubule inhibitors [Janssen et al., 2009]. This strategy might have two important advantages: (i) a partial SAC inhibition might be easier to achieve in clinical settings and (ii) low doses of microtubule inhibitors might be useful to minimize the unwanted inhibition of microtubule function in non-tumor cells. Low doses of, for example, taxanes lead to partial suppression of microtubule dynamics that clearly contribute to chromosome missegregation but not sufficient to induce apoptosis [Chen and Horwitz, 2002]. The novel conceptual idea is now to elevate the frequency of chromosome missegregation in those taxane-treated cells, which is expected to be no longer compatible with (tumor) cell survival. The latter can be achieved by a partial inhibition of the SAC. In fact, it has been demonstrated that a partial, non-lethal suppression of the spindle checkpoint kinases Mps1 or BubR1 can induce apoptosis in cancer cells treated with sub-lethal doses of taxol [Janssen et al., 2009]. Although this therapeutic concept has not yet been proven in vivo, it may open up an avenue for pre-clinical and clinical investigations combining low doses of conventional anti-mitotics with newly identified SAC inhibitors.

TARGETING THE CORRECTION MACHINERY FOR CHROMOSOME MAL-ATTACHMENTS

Another strategy to therapeutically elevate the frequency of chromosome missegregation could be the targeting of the mitotic machinery that is responsible for the correction of mal-oriented chromosomes. Mono-, syn-, and merotelic kinetochore attachments

occur naturally during the early phases of mitosis and are significantly elevated after transient anti-mitotic drug treatment, for example, after treatment and washout of the kinesin-5 inhibitor monastrol [Thompson et al., 2010]. The correction machinery involves in particular the centromeric Aurora-B kinase, which directly regulates kinetochore-based kinesin proteins with microtubule depolymerizing and thus, microtubule destabilizing activity. For instance, Kif2b and MCAK are non-motile kinesins involved in correcting chromosome mal-orientations and as such represent attractive novel drug targets [Howard and Hyman, 2007]. Inhibition of either Aurora-B or microtubule depolymerizing kinesins is expected to inevitably result in severe chromosome misalignment, which cannot be corrected and hence, is followed by the induction of apoptosis. Although being an attractive concept, no inhibitors for non-motile kinesins have been described so far.

TARGETING THE APC/C

Chemotherapeutic drugs that perturb mitotic spindle assembly including microtubule, Plk1, and kinesin-5 inhibitors activate the SAC leading to a mitotic arrest followed by the induction of apoptosis [Schmidt and Bastians, 2007]. In fact, it has been proposed that a pro-apoptotic signal accumulates during the SAC-mediated mitotic arrest that is important for the timely induction of apoptosis. On the other hand, premature exit from mitosis would prevent the initiation of apoptosis and promotes cell survival after drug treatment. Indeed, a compromised SAC, which allows slippage from the mitotic arrest, has been associated with drug resistance [Sudo et al., 2004; Kienitz et al., 2005]. Thus, apoptosis and mitotic slippage can be regarded as two competing pathways and shifting the balance between them determines whether a cell dies in mitosis, whether it exits from mitosis or whether it survives drug treatment [Gascoigne and Taylor, 2008]. These considerations have led to a novel therapeutic concept that relies on the maintenance of the mitotic arrest by inhibiting the APC/C mediated protein proteolysis (Fig. 1C). During a normal mitosis the APC/C is activated by a co-factor, known as Cdc20 that target cyclin B for its proteasome mediated degradation leading to the exit from mitosis. In the presence of unaligned chromosomes SAC proteins sequester Cdc20 and thus, prevent both, the degradation of cyclin B and the exit from mitosis. Consequently, loss of Cdc20 prevents the activation of the APC/C and causes a mitotic arrest [Musacchio and Salmon, 2007]. Importantly, Huang et al. [2009] have recently shown that the Cdc20 knockdown mediated mitotic arrest is sufficient to efficiently induce apoptosis in human cancer cells. As expected, mitotic cell death in this setup is neither dependent on spindle damage nor on the SAC [Huang et al., 2009]. As a result, blocking mitotic exit by preventing APC/C-mediated protein destruction might be a therapeutic strategy that circumvents both side effects due to microtubule disruption and resistance due to SAC defects or premature mitotic slippage. Data validating this concept in vivo are not available to date and it remains to be seen whether targeting Cdc20 or the APC/C can be achieved at high efficacy by the use of small molecule inhibitors.

TARGETING CANCER CELLS WITH SUPERNUMERARY CENTROSOMES

A frequent phenotype of human cancer cells is the appearance of supernumerary centrosomes, which can arise through unscheduled centrosome amplification. Since extra centrosomes correlate with CIN in human cancer it has long been assumed that multiple centrosomes cause multipolar mitotic spindles associated with multipolar chromosome segregation leading to highly aneuploid progenitors [Nigg, 2002]. However, most recent work has surprisingly demonstrated that multipolar chromosome segregations occurs very rarely and is typically associated with cell death. Instead, supernumerary centrosomes contribute to the formation of transient multipolar spindles that support the generation of chromosome mal-attachments including high rates of merotelic attachments [Ganem et al., 2009; Silkworth et al., 2009]. These transient spindle defects are followed by a clustering of the extra centrosomes at the poles allowing subsequently the execution of a bipolar chromosome segregation in anaphase. Hence, not the supernumerary centrosomes per se, but rather the chromosome mal-attachments represent a source of CIN in cancer cells with extra centrosomes. Nevertheless, the process of centrosome clustering might be an ideal point for therapeutic intervention. This concept involves the inhibition of regulators that are required for centrosome clustering leading to the induction of multipolar anaphases and cell death (Fig. 2). Indeed, a genome-wide genetic screen has identified the mitotic kinesin *KIFC1/HSET* as a gene required for centrosome clustering [Kwon et al., 2008]. Moreover, ablation of HSET induced multipolar anaphases and cell death specifically in cells with extra centrosomes and not in cells with the normal centrosome content. Like other mitotic kinesins, HSET might be a "druggable" target and hence, based on the results available to date, small molecule inhibitors for HSET are expected to provide a therapeutic window by targeting selectively cancer cells exhibiting centrosome amplification.

Interestingly, in addition to HSET, the SAC seems to be required for centrosomes clustering, probably providing extra time in mitosis to resolve the transient multipolar spindles [Kwon et al., 2008]. When inhibiting the SAC by small molecule inhibitors targeting Mps1 cancer cells with extra centrosomes are driven into catastrophic anaphases [Kwiatkowski et al., 2010]. This application of SAC inhibitors might provide an additional layer of tumor cell selectivity during anti-cancer therapy.

CONCLUSIONS

After decades of anti-cancer therapy using microtubule inhibitors the next generation of chemotherapeutics for novel mitotic targets are now eagerly awaited to be introduced into clinical application. However, it is uncertain whether these novel drugs targeting Plk1, kinesin-5/KSP, or Aurora kinases will fulfill all expectations. In fact, clinical trials for these anti-mitotics demonstrated efficacy, but in most cases no better than current microtubule inhibitors, albeit with improved side effect profiles [Jackson et al., 2007]. The major obstacle for microtubule drugs, significant neuropathies, is not observed for these new drugs. However, like microtubule inhibitors

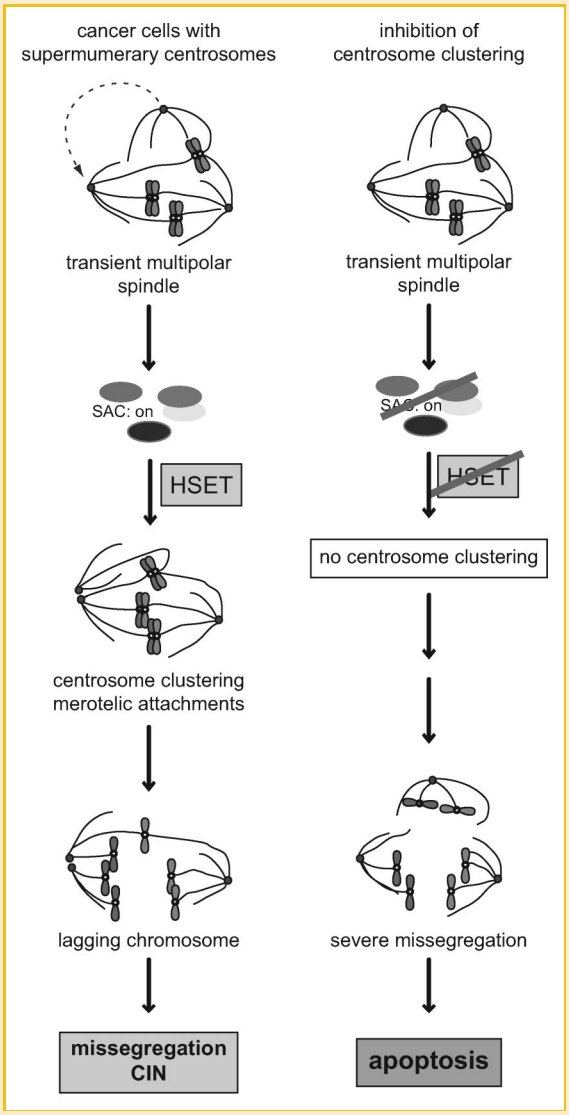

Fig. 2. Targeting cancer cell-specific centrosome clustering. Cancer cells with extra centrosomes exhibit the formation of transient multipolar spindles, which supports the generation of mal-oriented chromosomes. Before the onset of anaphase, centrosomes cluster at the poles and this requires the mitotic kinesin HSET as well as the SAC, which provides extra time in mitosis. Centrosome clustering allows the formation of bipolar anaphase spindles and chromosome segregation, albeit with reduced fidelity resulting in chromosomal instability (CIN) and aneuploidy. Inhibition of HSET or the SAC prevents centrosome clustering leading to multipolar anaphases and chromosome segregation, which typically results in cell death. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

drugs targeting mitotic kinases or kinesins do not provide a great layer of tumor cell selectivity. Therefore, a mitotic drug target providing the absolute tumor cell selectivity is still to be identified. Maybe targeting the SAC or inhibiting centrosome clustering will hold the premise for being an ideal anti-mitotic drug target.

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