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REVIEW

The spindle assembly checkpoint: perspectives in tumorigenesis and cancer therapy

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Abstract **Loss or gain of chromosomes, a condition known as aneuploidy, is a common feature of tumor cells and has therefore been proposed as the driving force for tumorigenesis. Such chromosomal instability can arise during mitosis as a result of mis-segregation of the duplicated sister chromatids to the two daughter cells. In normal cells, mis-segregation is usually prevented by the spindle assembly checkpoint (SAC), a sophisticated surveillance mechanism that inhibits mitotic exit until all chromosomes have successfully achieved bipolar attachment to spindle microtubules. Complete abrogation of SAC activity is lethal to normal as well as to tumor cells, as a consequence of massive chromosome mis-segregation. Importantly, many human aneuploid tumor cells exhibit a weakened SAC activity that allows them to tolerate gains or losses of a small number of chromosomes; and interfering with this SAC residual activity may constitute a suitable strategy to kill cancer cells. This review focuses on the potential link between SAC and tumorigenesis, and the therapeutic strategy to target the SAC for cancer treatment.**

Keywords spindle assembly checkpoint, mitosis, chromosome instability, tumor, cancer therapy

Introduction

The cell cycle is a ubiquitous and complex process of highly coordinated events whereby cells grow and proliferate, leading to development of all living organisms and to regeneration of adult tissues (Schafer, 1998). Mitosis is a particularly important phase of the cell cycle, since it ensures accurate segregation of the genetic material, duplicated at the S-phase, into the two daughter nuclei, therefore having a remarkable contribution to normal cell division and proliferation. The orderly progress through the cell cycle is regulated by specific checkpoints – control loops that make the beginning of each event dependent on the proper accomplishment of the previous one (Clarke and Giménez-Abián, 2000). Four checkpoints are superimposed on the cell cycle: DNA damage checkpoints occurring at G1/S, S and G2/M, and the mitotic checkpoint. These checkpoints coordinately ensure that the cell fulfils every requirement to go through the next stage and are able to block cell cycle if those requirements are not met (Tyson and Novak, 2008).

The mitotic checkpoint or spindle assembly checkpoint (SAC) is the mechanism that is specifically in charge for the control of mitosis. It consists of a complex signaling cascade that is constitutively activated as soon as cells enter mitosis and is responsible for cell cycle blockage at the metaphase-to-anaphase transition if there are non-attached or mis-attached kinetochores to microtubules, if chromosomes are not correctly aligned at the metaphase plate, or if chromosome-microtubule attachments do not generate the appropriate bipolar tension (Musacchio and Salmon, 2007). When this happens, the cell is given some time to correct the error. If the error is adequately solved, the SAC is eventually silenced and the cell progresses into anaphase; otherwise, the cell is committed to die via apoptosis (Niikura et al., 2007).

Although the number of components involved in the SAC molecular pathway is still growing, a specific set of proteins comprising Mad1, Mad2, Mad3/BubR1, Bub1, Bub3, and Mps1 constitutes the bona fide SAC proteins responsible for the generation of the inhibitory "wait anaphase" signal that prevents premature sister chromatid separation (Fig. 1) (Hoyt

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et al., 1991; Earnshaw and Mackay, 1994; Roberts et al., 1994; Bharadwaj and Yu, 2004; Cheeseman and Desai, 2008; Logarinho and Bousbaa, 2008; Kops, 2009). Sister chromatids are held together at the centromere region by a complex of cohesin proteins, which can be degraded by the proteolytic activity of the enzyme Separase. Because it destroys the molecular connection between sister chromatids, Separase is required for their separation and, thus, for their movement toward opposite poles at anaphase. Separase is under the control of Securin. When bound to Separase, Securin prevents it from cleaving cohesins, thus delaying the onset of anaphase. In turn, Securin levels are regulated by the anaphase-promoting complex/cyclosome (APC/C), a multi-subunit E3 ubiquitin ligase that targets proteins for degradation by the 26 S proteasome (Morgan, 1999; Reddy et al., 2007; Stegmeier et al., 2007). Upon APC/C ubiquitination, Securin is degraded, thus leading to the activation of Separase which cleaves one of the cohesin's subunits, Scc1. Sister chromatids are then able to segregate on the mitotic spindle and anaphase takes place (Fig. 1) (Nasmyth, 2005; Bannon and Mc Gee, 2009; Przewloka and Glover, 2009). APC/C also targets Cyclin B for degradation, which inactivates cyclin-dependent kinase 1 (Cdk1) thereby promoting mitotic exit.

APC/C ubiquitin ligase activity is dependent on the binding of Cdc20, which is the principal SAC target. Unattached kinetochores recruit Mad2, Bub3 and Mad3/BubR1 to form the mitotic checkpoint complex (MCC), the SAC inhibitory signal (Fig. 1) (Sudakin et al., 2001). By sequestering Cdc20, the MCC keeps the APC/C inactive.

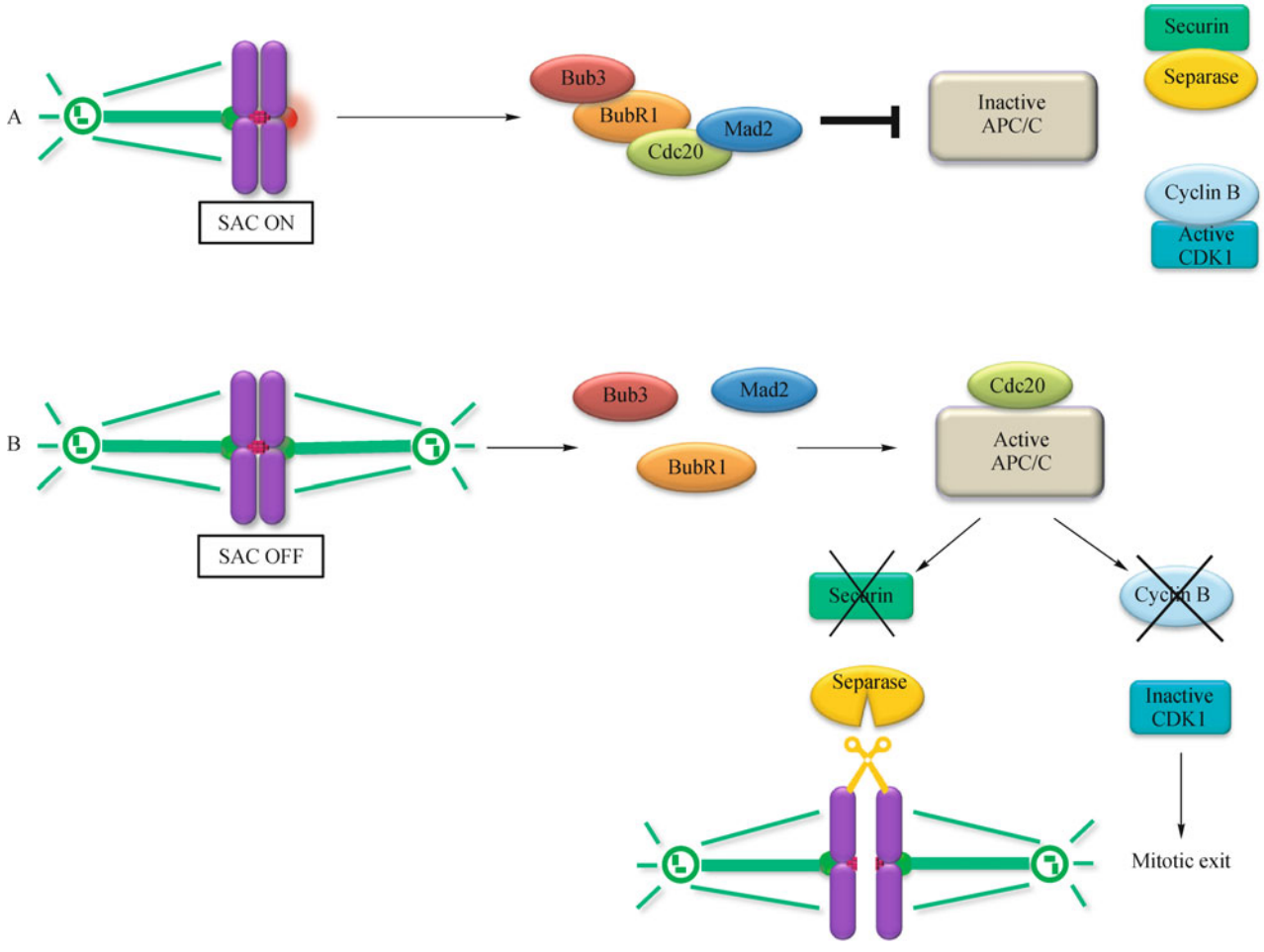

Figure 1 Molecular basis of spindle assembly checkpoint (SAC) signaling. A: The SAC is activated by the presence of unattached kinetochores (red circle). Unattached kinetochore recruits SAC proteins to generate the Mitotic Checkpoint Complex (MCC), the diffusible inhibitory signal composed of Mad2, Mad3/BubR1, and Bub3. By sequestering Cdc20, the MCC keeps the APC/C inactive, preventing it from targeting Securin and Cyclin B for degradation; this way, sister-chromatid cohesion is maintained and the cell cycle is arrested, respectively. B: When all kinetochores have aligned, the MCC disassembles and Cdc20 is released. Cdc20 activates the APC/C, leading to Securin and Cyclin B degradation. Securin degradation leads to the activation of the protease Separase, which cleaves cohesin, leading to sister-chromatid separation. Cyclin B degradation decreases the cyclin-dependent kinase (Cdk) 1 activity, which results in mitotic exit. Thus, by stabilizing Securin and Cyclin B, the SAC prevents premature sister-chromatid separation in the presence of unattached kinetochores, and maintains the mitotic state, respectively.

Once the last kinetochore becomes successfully attached, the MCC disassembles and is no longer produced. Microtubule minus-end motor Dynein contributes to this process by promoting SAC component stripping away from the kinetochore (Howell et al., 2001). Cdc20 becomes free to bind and activate APC/C which, by triggering Securin and Cyclin B degradation, promotes anaphase onset.

The main goal of the equal segregation of the duplicated chromosomes during mitosis is the correct distribution of genetic information to daughter cells. Although this process is tightly regulated by the spindle assembly checkpoint, errors may occur resulting in chromosome loss or gain in daughter cells, a phenomenon known as aneuploidy (Suijkerbuijk and Kops, 2008). It is known that there is a causal connection between genome mis-segregation, genetic instability and premature aging, inherited diseases and predisposition to cancer (Aguilera and Gómez-González, 2008). In fact, most solid tumors are aneuploid and many mis-segregate chromosomes at very high rates, thus showing chromosomal instability (CIN). These findings suggest that aneuploidy and CIN may contribute to tumorigenesis, but their exact role in this process is still an unresolved issue (Chi and Jeang, 2007; Suijkerbuijk and Kops, 2008; Bannon and Mc Gee, 2009; Dalton and Yang, 2009). Although aneuploidy may represent a proliferative disadvantage, since abnormal mitotic divisions result in chromosome aberrations that are incompatible with cell viability, many tumor cell lines are aneuploid, pointing toward the existence of a complex relationship between CIN and carcinogenesis (Weaver et al., 2007). The exact mechanism that lies behind these chromosomal imbalances is not precisely established, but it is more likely the combination of defects in apoptosis-related machinery and in the processes that monitor chromosome segregation during mitosis, one of them being the spindle assembly checkpoint (Kops et al., 2005; Schmidt and Medema, 2006). Taking into account the role of SAC in keeping cells from prematurely segregating their genetic material, its activity is crucial to guarantee the fidelity of mitotic events, to prevent genomic imbalance and the abnormal cell proliferation that characterizes tumor tissue. Indeed, many cancer cells exhibiting aneuploidy were reported to have a compromised SAC (Bharadwaj and Yu, 2004), suggesting an association between deregulated levels or function of SAC components and CIN (Fig. 2). It was also proposed that cells with defective SAC activity have a higher carcinogenic potential than those with an efficient SAC (Chi and Jeang, 2007; Suijkerbuijk and Kops, 2008; Bannon and Mc Gee, 2009; Dalton and Yang, 2009). In the next sections, we will focus on

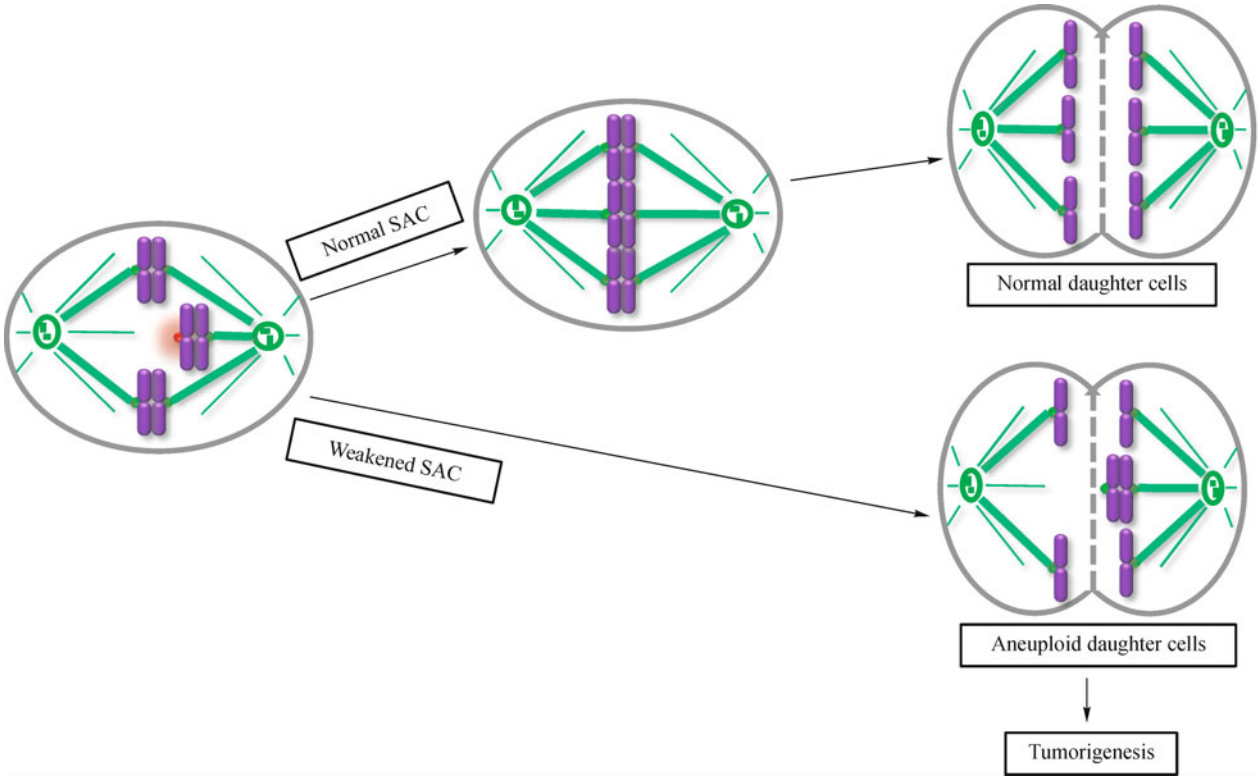

Figure 2 Defects in SAC activity can contribute to aneuploidy and tumorigenesis. Chromosome mis-segregation is prevented in cells with a fully functional SAC. In cells with weakened SAC activity, the residual checkpoint activity can still ensure the accuracy of chromosome segregation. However, occasional mis-segregations may escape from SAC control and lead to aneuploidy, which may contribute to tumorigenesis.

the underlying molecular basis of SAC deficiency in tumor cells and how this information can be used in cancer therapy.

Mutations in SAC genes are rather rare in tumor cells

The fact that CIN is a common feature in tumor cells led to the suspicion that mutations in genes encoding for their SAC proteins could underlie their aneuploidy. Several studies were carried out in order to match distinct neoplasies with SAC gene mutations. Surprisingly, few studies reported mutations in human SAC genes. Mutations in *MAD1L1* gene were reported in a large panel of 44 cancer cell lines and 133 primary cancer samples (Tsukasaki et al., 2001). Mutations and deletions in both *bub1* and *bubr1* genes were also seen in four cases of adult T cell leukemia/lymphoma and in a number of B cell lymphomas (Ohshima et al., 2000). Diverse mutations in *bubr1*, *mad2* and *mad1* genes were detected in studies with colon and breast cancer lines (Cahill et al., 1999; Myrie et al., 2000), while a mutation in *bub1* gene was identified in primary lung cancer (Gemma et al., 2000).

However, these mutations alone do not account for the loss of heterozygoty and aneuploidy that are observed in some tumors. In fact, several other studies were unable to detect mutations in any SAC gene, which strongly indicates that they may not be the major cause of tumorigenesis (Cahill et al., 1999; Imai et al., 1999; Takahashi et al., 1999; Yamaguchi et al., 1999; Gemma et al., 2000; Myrie et al., 2000; Sato et al., 2000; Weitzel and Vandré, 2000; Gemma et al., 2001; Haruki et al., 2001; Hernando et al., 2001; Mimori et al., 2001; Olesen et al., 2001; Reis et al., 2001; Shigeishi et al., 2001b; Ouyang et al., 2002; Tanaka et al., 2008).

Tumor cells frequently show altered expression levels of SAC components

Besides gene mutations, many other mechanisms may result in aneuploidy. For instance, an increase in the number of centrosomes generates CIN by promoting multipolar anaphase, a highly abnormal division phenotype that produces three or more aneuploid daughter cells (Ganem et al., 2009). Incorrect microtubule-kinetochore attachments are responsible for a dramatic increase in mitotic abnormalities (Green and Kaplan, 2003; Bakhoum et al., 2009; Silkworth et al., 2009). Lastly, molecular defects that weaken the SAC response induce aneuploidy by causing premature chromosome segregation (Kops et al., 2005). Because only a limited number of cancer cell types were shown to display SAC gene mutations, research in this area started to focus on the detection of changes in the expression levels of its components, at the gene and protein levels. Even relatively minor changes of this nature were later shown to promote tumorigenesis (Bharadwaj and Yu, 2004).

SAC efficiency can be easily evaluated by determining the ability of a cell population to sustain a chronic mitotic arrest in response to compounds that disrupt microtubule function. The status of the SAC and the underlying molecular changes have been investigated in various tumor cell lines and clinical samples of divergent origins, such as lung cancer (Masuda et al., 2003), hepatocellular carcinoma (Saeki et al., 2002), oral cancer (Mondal et al., 2007), head and neck squamous cell carcinoma (Minhas et al., 2003) and breast cancer cell lines (Yoon et al., 2002), among others. Overall, these studies showed that SAC impairment occurs with a high frequency in many cancer cells, and, as stated below, this SAC impairment is often associated with altered expression in SAC components. However, it remains to be proven whether the alterations in SAC genes, whether by mutation or by altered levels of their expression, are directly responsible for SAC weakening.

SAC is thought to be essential for ensuring accurate chromosome segregation by imposing mitotic delay in response to a spindle defect. Complete ablation of SAC activity was shown to be lethal in several studies (Basu et al., 1998; Kops et al., 2004). Mad2 knock out leads to rapid cell division and to premature anaphase, leading to mitotic catastrophe and apoptosis (Dobles et al., 2000; Orr et al., 2007; Wang et al., 2010). The downregulation of the key component of the spindle checkpoint BubR1 causes an increase in polyploidy in murine embryonic fibroblast cells and enhanced megakaryopoiesis in bone marrow progenitor cells (Scannevin et al., 2004); BubR1 haploinsufficiency mice and RNA interference-mediated downregulation of this protein result in rapid tumor development (Dai et al., 2004). Downregulation of Mad1, in turn, causes SAC inactivation and aneuploidy (Kienitz et al., 2005), while reduced expression of Mad2 and Bub1 proteins is associated with spontaneous abortions (Shi et al., 2010). Loss of Bub1 below a critical threshold causes chromosome mis-segregation and can lead to spontaneous tumorigenesis (Basu et al., 1999; Jeganathan et al., 2007). Bub3 haploinsufficient cells in mice show increased aneuploidy and premature sister chromatid separation (Kalitsis et al., 2005).

In colon cancer, *mad2l2* gene overexpression by 3- or more folds has been positively correlated with reduced patient survival (Rimkus et al., 2007). *bub1*, *bubr1*, *mad1* and *mad2l1* genes showed significant expression differences in clear cell kidney carcinomas when compared to controls, suggesting that their overexpression plays a relevant role in cytogenetic and morphologic progression of kidney carcinomas (Pinto et al., 2008). The altered expression levels in *mad1*, *mad2l1*, and *mad2l2* genes contribute significantly to renal carcinogenesis, characterized by multiple numerical chromosome abnormalities and a defective SAC response (Pinto et al., 2007). The same happens in nasopharyngeal carcinoma cells (Wang et al., 2000), colorectal cancers (Burum-Auensen et al., 2008), breast cancer cell lines (Percy et al., 2000), testicular germ cell tumor (Fung et al., 2007),

and gastric cancer cells (Wang et al., 2010). Similar results were reported concerning the loss of SAC function, but associated with reduced levels of Bub1 in colon carcinoma and in acute myeloid leukemia samples (Lin et al., 2002; Burum-Auensen et al., 2008). High *bubr1* expression was detected in 25.9% of subjects with salivary duct carcinoma (SDC), and high *mad2l1* expression in 55.6% of them. However, survival analysis failed to show prognostic significance for SAC gene expression in SDCs, as well as for the other markers under analysis (Ko et al., 2010). The expression of *bubr1* was studied by immunohistochemistry in 181 gastric cancer samples and by real-time PCR in several gastric cancer cell lines. High *bubr1* expression was found in 50.3% of the samples analyzed, correlating significantly with DNA aneuploidy, tumor invasiveness, lymph node metastasis, liver metastasis, and poor prognosis (Ando et al., 2010). *bub1*, *bubr1*, and *mad2* were shown to be overexpressed in thyroid carcinomas with aggressive nature (Wada et al., 2008). The SAC genes *mad2* and *bubr1* were also found to be overexpressed in esophageal squamous cell carcinoma cell lines. Interestingly, a decrease in their expression was proven to increase their sensitivity to the anti-microtubule drugs paclitaxel and docetaxel (Tanaka et al., 2008). Downregulation of *mad2* was associated with defective SAC in 11 hepatoma cell lines (Sze et al., 2004). Bub1 protein expression levels were significantly high in gastric tumors (Grabsch et al., 2004). *bubr1* expression was found to be upregulated in human lung cancers (Seike et al., 2002). Several other studies have documented changes in expression levels of SAC genes and their proteins (Yamaguchi et al., 1999; Myrie et al., 2000; Percy et al., 2000; Shigeishi et al., 2001a; Saeki et al., 2002).

In addition, it is noteworthy that changes in the expression levels of these proteins are likely to affect other cell activities besides mitosis, since they are expressed throughout the cell cycle and are implicated in several other processes, such as apoptosis (BubR1), transcriptional repression (Bub3), DNA replication checkpoint (Mad2), and gross chromosomal rearrangements (Mad2, Bub3, BubR1) (Kops et al., 2005). Efficient SAC activity is based on the fragile equilibrium between its components and their expression levels, which can be exploited envisaging a potential use in cancer therapy.

Targeting SAC for anti-cancer therapy

The build-up of knowledge regarding the mechanisms responsible for cell cycle progression and division made it possible to design pharmacological approaches to specifically interfere with cancer cell proliferation.

Given the role of microtubules and the associated proteins in mitotic spindle assembly, chromosome segregation, and cell division, they became suitable targets of cancer treatment strategies. Chemotherapeutic approaches that make use of microtubule-targeting agents (MTAs) have been used with a considerable degree of success in a wide range of tumor types (Bannon and Mc Gee, 2009). Vinca alkaloids and taxanes like paclitaxel and docetaxel are among the most widely used anti-microtubule drugs. By interfering with microtubule dynamics, theses drugs cause long-term SAC-dependent mitotic arrest and cell death, which compromises tumor cell proliferation, making them valuable anti-cancer tools (Jordan and Wilson, 2004). Parameters like their concentration, time of exposure and type of target cell influence their effectiveness, but the mechanisms by which prolonged mitotic arrest is connected with cell death are not completely understood yet (Bannon and Mc Gee, 2009).

However, because many tumor cells have defective SAC that do not fully respond to mitotic errors such as lack of kinetochore attachment, microtubule-interfering drugs do not have the desired complete effectiveness (Bolanos-Garcia, 2009). Therefore, optimization of novel therapeutic approaches that selectively interfere with SAC proteins or other components of the SAC pathway would be useful. For instance, the use of inhibitors that specifically interfere with Aurora B kinase, an enzyme involved in correcting chromosome mis-attachments, sharply decreases viability of rapidly dividing cells, resulting in a 98% reduction in tumor volume in nude mice injected with human leukemia cells (Bolanos-Garcia, 2009). In turn, the inhibition of KSP (kinesin motor protein), which is crucial for centrosome separation and spindle assembly, was shown to have a considerable anti-proliferative effect on tumor cells that were resistant to taxanes (Bannon and Mc Gee, 2009). Inhibition of Cdc20 would be of special interest given its role as the APC/C modulator. However, the analysis of its effects is complex because of its interactions with other checkpoint components (Bolanos-Garcia, 2009).

The decrease in BubR1 or Mad2 protein levels in human cancer cells or the inhibition of BubR1 kinase activity were known to be responsible for apoptotic cell death within six divisions, except when cytokinesis was also inhibited. Thus, the suppression of SAC signaling is invariably lethal as a consequence of massive chromosome loss, a finding that has implications in the inhibition of tumor cell proliferation (Kops et al., 2004; Michel et al., 2004). While an impaired SAC can lie beneath an increased frequency of aneuploidy, a more severe weakening of this mechanism culminates in cell death. Since protein kinases are well-validated targets for drug development, those that are known to function in the SAC, namely Bub1, BubR1 and Mps1, are among the suitable drug targets (Bolanos-Garcia, 2009). Targeting the essential components of SAC, such as BubR1 and Bub1 kinases, may preferentially kill mitotic checkpoint-deficient tumor cells. Several small molecule compounds are being developed based on the protein–protein interface structure and by high-throughput screens to identify new compounds that disrupt SAC function (Pan et al., 2008; Bolanos-Garcia, 2009; Screpanti et al., 2010; Zeng et al., 2010). One important form of drug regimen that could lead to mitotic catastrophe is siRNA (small interfering RNA)-mediated knockdown against

SAC proteins, such as Mad2 and BubR1, which suppresses cell proliferation and induces apoptosis. However, systemic injection of siRNA presents some limits due to their short half-life, vulnerability to degradation, high toxicity and tendency to trigger an immune response (Bagasra and Prilliman, 2004). In this context, the use of colloidal carriers such as nanoparticles or therapeutic polymers represents an attractive alternative because of its potential to induce controlled release of siRNAs in target tissues (Satchi-Fainaro and Duncan, 2006; Ji et al., 2009). Considering the recent advances in chemistry and delivery strategies, therapeutic potential of RNA interference strategies targeting components of the SAC pathway opens exciting possibilities for the future of cancer therapy.

Conclusion

In recent years, major advances have been made in the understanding of the mechanisms that regulate mitotic progression and guarantee genomic stability and several studies reported alterations in SAC activity and in expression levels of SAC components as a widespread property of tumors. Because SAC silencing generates karyotypic abnormalities that are incompatible with cell viability and drives cells into apoptosis, targeting SAC components in a way that will preferentially kill cancer cells with leaky checkpoint remains an attractive challenge for researchers in the anti-cancer therapy field. Considering the recent advances in chemistry and delivery strategies which are currently in development, therapeutic potential of small molecules and siRNAs against SAC complexes opens exciting possibilities for the future of cancer therapy.

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