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Anti-mitotik İlaçla Tetiklenen Uzun Süreli Mitotik Areste Hücresel Yanıtın Çeşitliliği

Variation in Cellular Responses to Anti-mitotic Drug-Induced Prolonged Mitotic Arrest

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Özet

Günümüzde kanser tedavisinde başarıyla kullanılan anti-mitotik ilaçlar, mikrotübülleri hedef alarak "İğ İpliği Kontrol Noktası (İKN)" nın kronik aktivasyonunu tetikler. Anti-mitotik ilaçlarla tetiklenen kronik İKN aktivasyonu, bu güne kadar test edilen tüm hücre hatlarında uzun süreli mitotik-areste yol açmıştır. Ancak, uzun süreli mitotik areste karşı oluşan tek tip bir hücresel cevap bulunmamaktadır. Diğer bir deyişle, hücreler uzun süreli mitotik aresti takiben farklı post-mitotik kaderlere maruz kalabilirler. Bu nedenle, farklı kanser tiplerindeki hücreler, hatta farklı hastalara ait aynı kanser tipindeki hücreler anti-mitotik ilaçlara gösterdikleri hassasiyet açısından büyük farklılıklar gösterebilirler. Bu ilaçlara karşı gösterilen hücresel cevaplardaki çeşitlilik kanser tedavisinde ciddi bir sorun teşkil etmektedir. Bu nedenle, uzun süreli mitotik aresti takiben hangi hücre kaderine teslim olunacağı kararının moleküler temellerinin iyi anlaşılması kanser tedavisinde daha başarılı stratejiler geliştirebilmek için oldukça önemli bilgiler sağlayabilir. Bu derlemede, İKN'yi anti-mitotik ilaçlarla aktive olan bir sinyal yolağı olarak ve kanserdeki rolü açısından değerlendirdik. Ayrıca, anti-mitotik ilaçlarla tetiklenen uzun süreli mitotik aresti takip eden farklı hücre kaderlerinin neler olduğunu ve bunların arasından hücrelerin nasıl belirli bir kadere teslim olduğunu açıklayan yeni bir modeli tartıştık.

Anahtar Kelimeler: Anti-mitotik ilaçlar, İğ ipliği kontrol noktası, Kanser, Hücre kaderi.

Abstract

Anti-mitotic drugs, which are successfully used in cancer treatment today, trigger chronic activation of the "Spindle Assembly Checkpoint (SAC)" by targeting microtubules. Chronic activation of the SAC induced by anti-mitotic drugs causes a prolonged mitotic arrest in all cancer cell lines tested. However, there is not a single cellular response to the prolonged arrest. In other words, cells can undergo different fates following the prolonged arrest. Therefore, cells from different cancer types, even cells from the same cancer type in different patients may differ greatly by their susceptibilities to anti-mitotic drugs. The variation in cellular responses to these drugs presents a serious problem in cancer treatment. Therefore, understanding the molecular basis of the cell fate determination following the prolonged arrest might provide important information to develop more successful strategies in cancer treatment. Here, we review the SAC as a pathway activated by anti-mitotic drugs as well as its role in cancer. We also discuss the different cell fates following the prolonged arrest induced by these drugs and describe a recently proposed model to explain how cells may commit to a certain cell fate.

Keywords: Anti-mitotic drugs, Spindle assembly checkpoint, Cancer, Cell fate.

The Spindle Assembly Checkpoint (SAC)

Mitosis is a cell cycle phase during which cells segregate their genetic material (karyokinesis) and distribute cellular organelles (cytokinesis) into two daughter cells. Sister kinetochores (KTs) have to attach spindle microtubules (MTs) emanating from opposing poles of the mitotic spindle (bipolar attachment) for accurate chromosome segregation in mitosis. Unequal chromosome segregation during mitosis is one of the major causes of aneuploidy, a common characteristic of cancer cells (1). Spindle assembly checkpoint (SAC) is an evolutionarily conserved surveillance mechanism that monitors the status of KT-MT attachments in metaphase to ensure the fidelity of chromosome segregation. SAC is crucial for the regulation of metaphase to anaphase transition; it delays the anaphase onset until each KT is properly attached to MTs, ensuring accurate chromosome segregation, thus genomic stability. SAC gets activated in the presence of either unattached or improperly attached KTs and arrests the cell in metaphase until the erroneous attachment(s) are corrected

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(2). Even a single unattached (3) or a single improperly attached KT (4) is sufficient to activate the SAC.

Two independent genetic screens in Saccharomyces cerevisiae identified two groups of SAC genes whose mutations caused inability to arrest in mitosis in response to MT depolymerizing drugs. SAC genes include the MAD (mitotic arrest deficient) genes MAD1, MAD2, MAD3 and the BUB (budding uninhibited by benzimidazole) genes BUB1, BUB2, BUB3. In addition, an essential protein kinase encoded by MPS1 (monopolar spindle), which was originally identified by its function in spindle pole body duplication, has later been identified as another SAC component (2). Loss-of-function mutations in SAC genes result in genomic instability by allowing chromosome segregation in anaphase before all chromosomes establish proper KT-MT attachments (5).

Although the SAC genes are evolutionarily well conserved among eukaryotes, there are some differences in their KT localization patterns and their requirements for viability. SAC is dispensable for viability in S.cerevisiae under normal growth conditions, but it is essential in vertebrates (6, 7, 8). Moreover, all SAC genes are recruited to KTs during normal mitoses in animal cells, whereas in S.cerevisiae, Bub1 and Bub3 bind to KTs in early stages of normal mitosis in a cell cycle regulated manner, whereas Mad1 and Mad2 are recruited to KTs only when there is a spindle damage interfering with KT-MT attachments as assayed by CHIP (9).

Improper Kinetochore (KT) – Microtubule (MT) Attachments Activate the SAC

Attachments between microtubules (MTs) and kinetochores (KTs) are monitored by the SAC to ensure accurate chromosome segregation in mitosis. MTs are cytoskeletal polymers that play an essential role in chromosome segregation during mitosis. They are composed of 13 protofilaments arranged laterally around a hollow core. Each polymer consists of head-to-tail arrays of α/β-tubulin heterodimers. Therefore, MTs are polarized structures with a fast-growing, β-tubulin exposed plus end and a slow-growing, α-tubulin exposed minus end (10). MTs are highly dynamic structures and switch between phases of growth and shrinkage rapidly, a process known as “dynamic instability” (10). Polarized and dynamic structure of MTs is crucial for accurate chromosome segregation during cell division. KTs are multiprotein complexes assembled on centromeric DNA. They mediate attachments between chromosomes and plus ends of the MTs. Accurate chromosome segregation in mitosis requires “bi-orientation” of sister chromatids. Sister chromatids must attach to MTs emanating from opposing spindle poles in order to achieve chromosome bi-orientation (11).

During prometaphase, dynamically unstable MTs make associations with KTs by a process called “search and capture”. During this process, MT plus ends grow and shrink from the spindle poles until they are captured by a KT. Once a stable end-on attachment between a sister KT and MT(s) is established, the other sister KT soon captures a MT emanating from the opposite spindle pole, resulting in the bi-orientation of the chromosome (12). Besides these correct, “bipolar (amphitelic) attachments”, aberrant MT attachments can also be observed including “monotelic attachments” where only one of the sister KTs binds to MTs, “syntelic attachments” where both sister KTs attach MTs emanating from the same spindle pole and “merotelic attachments” where one sister KT binds to MTs extending from opposing spindle poles (13) (Figure 1). Bipolar attachments are stabilized, whereas improper attachments are selectively destabilized, providing an opportunity to establish correct (bipolar) attachments.

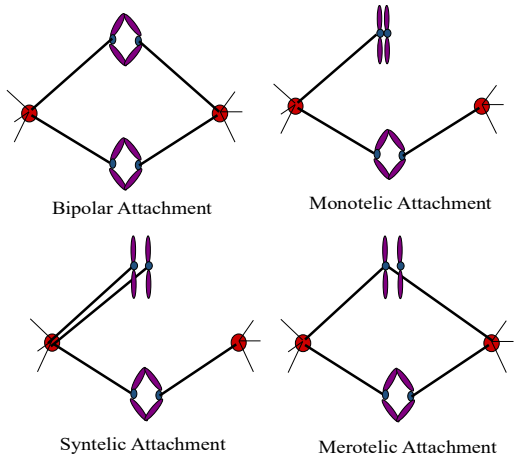

Figure 1. *Kinetochore-Microtubule Attachments*
In bipolar attachments each sister kinetochore binds to microtubules emanating from the opposing cell poles. In monotelic attachments, only one sister kinetochore binds to microtubules, in syntelic attachments both sister kinetochores attach microtubules extending from the same spindle pole and in merotelic attachments one sister kinetochore binds to microtubules emanating from opposing spindle poles.

Improper attachments would lead to unequal chromosome segragation, thus aneuploidy, if left uncorrected. Therefore, KT-MT attachments must be carefully monitored to prevent tumorigenesis, since aneuploidy has been shown to be a hallmark of cancer cells (14).

Although, it is universally agreed upon that the spindle checkpoint monitors the state of KT-MT attachments, the exact nature of the primary SAC-activating signal produced from the unattached or improperly attached KT(s) remains controversial. Several experiments in different organisms suggest two possible spindle checkpoint activating signals produced by the KT in the presence of attachment defects: lack of tension across the sister KTs (tension model) and lack of MT attachment at the KT (attachment model) (15). Distinguishing between the two models is difficult because of the interdependence between tension and attachment. Tension relies on attachment, because KTs have to bipolarly attach spindle MTs to generate tension (16). Attachment is also affected by tension, based on the observation that applying tension with a micromanipulation needle both stabilizes and increases the number of attachments (17).

Bipolar attachments are essential to exert tension across sister KTs. Because, these attachments generate the pulling forces opposing the cohesive forces that hold sister chromatids together. Tug-of-war between the two opposing forces creates tension across the sister KTs (18), which can be visualized by an increased distance between sister KTs (15). Experimental evidence for the “tension model” is provided by a study carried out in praying mantis. Shortly after applying tension to a free X chromosome (which normally delays metaphase up to 7 hours) by a microneedle, the cell entered anaphase, indicating that restoring tension is sufficient to satisfy the SAC (4). On the other hand, selective destruction of the unattached KT by laser ablation has been shown to abrogate the SAC. Since the checkpoint is not activated in the laser ablated KT that is not under tension, these data suggest that the checkpoint responds to lack of MT attachments (18).

Another possible model for the SAC activation is that the SAC senses both signals via two separate branches. Consistent with this model, vertebrate and yeast studies revealed that there is differential KT localization of the SAC proteins in response to lack of tension or lack of attachment; Mad2 localizes to KTs in response to lack of KT-MT attachment rather than lack of tension, whereas Bub1 and BubR1 are recruited specifically to tension defective KTs (19). These data suggest that different SAC proteins might be monitoring different aspects of KT-MT attachments. A more recent study in budding yeast genetically separated the two signals and provided experimental data for the SAC sensing both signals via two distinct branches of the SAC and identified three KT proteins that are uniquely involved in the attachment branch (20). Determining the attachment dependent

regulation of these KT proteins will provide critical information on the signaling cascade involved in the attachment branch of the SAC. The more we know about the activation and signaling of the SAC pathway(s), the better we will understand how the primary signal is amplified in a way that only a single improperly attached KT is sufficient to activate the SAC and arrest the cell in metaphase.

SAC Activity and Mitotic Arrest

Sister chromatids are held together by a ring-shaped, evolutionarily conserved protein complex called "cohesin" composed of four subunits: Smc1, Smc3, Scc1/Mcd1, and Scc3. Cohesin initially binds to DNA during DNA replication in eukaryotes, entraps sister chromatids and generates cohesion. Cohesion between sister chromatids has to be removed to enable chromosome segregation in anaphase. Once every KT bipolarly attaches to spindle MTs, separase initiates anaphase by cleaving the cohesin subunit Scc1/Mcd1 from chromosomes. The "anaphase inhibitor", securin, binds to and inhibits separase until the anaphase onset. The key step for anaphase initiation is the securin proteolysis by an E3 ubiquitin ligase called the anaphase promoting complex or cyclosome (APC/C) (Figure 2). APC/C targets several proteins through associating with different substrate specific co-activators. One of these co-activators, Cdc20 targets APC/C for securin and cyclin B degradation (2).

Data from yeast, frog and mammalian studies demonstrated that the ultimate target of the SAC is the essential APC/C co-activator, Cdc20. SAC proteins BubR1/Mad3, Bub3, Mad2 form a complex with Cdc20, called the mitotic checkpoint complex (MCC), which inhibits the activity of APC/C by directly binding to it. It has been proposed that although the MCC is present throughout the cell cycle, it cannot inhibit APC/C; it is capable of APC/C inhibition only in the presence of unattached KTs (21). In the presence of improperly attached or unattached KTs, MCC inhibits APC/C. APC/C inhibition prevents securin degradation, thus separase activation. Therefore, chromosomes cannot be separated and the cell remains in metaphase (Figure 2). Once every chromosome establishes bipolar attachments, SAC activity must be extinguished for mitosis to proceed. Extinguishing the SAC activity involves disassembly of the APC/C inhibitory MCC. As a result of MCC disassembly, Cdc20 becomes free to activate the APC/C, which targets securin and cyclin B for degradation, leading to chromosome segregation and exit from mitosis (2).

SAC Activity and Cancer

The ultimate purpose of cell division is to accurately replicate the genetic material and then equally segregate it into two daughter cells. Failure of parental cells to properly segregate their chromosomes in mitosis may lead to genetic instability in daughter cells. Genomic instability, described as an increased tendency of genetic alterations, is commonly observed in solid tumors and plays a major role in tumorigenesis (22). A type of genomic instability, chromosomal instability (CIN) - known as aneuploidy - indicates numerical changes in chromosomes and has been considered as a common characteristic of tumor cells (about 90% of solid tumors and 85% of hematopoietic neoplasias are known to be aneuploid) (14). Since precise chromosome segregation, ensured by the functional SAC activity, is a major mechanism to minimize the risk for aneuploidy, it has been suggested that SAC dysfunction may be responsible for aneuploidy in human cancers. Consistent with this, a large number of CIN colorectal cancer cell lines have been reported to have a defective SAC activity (23). Besides, several studies in animal models reported that alterations in SAC genes might lead to aneuploidy and induce tumorigenesis (24).

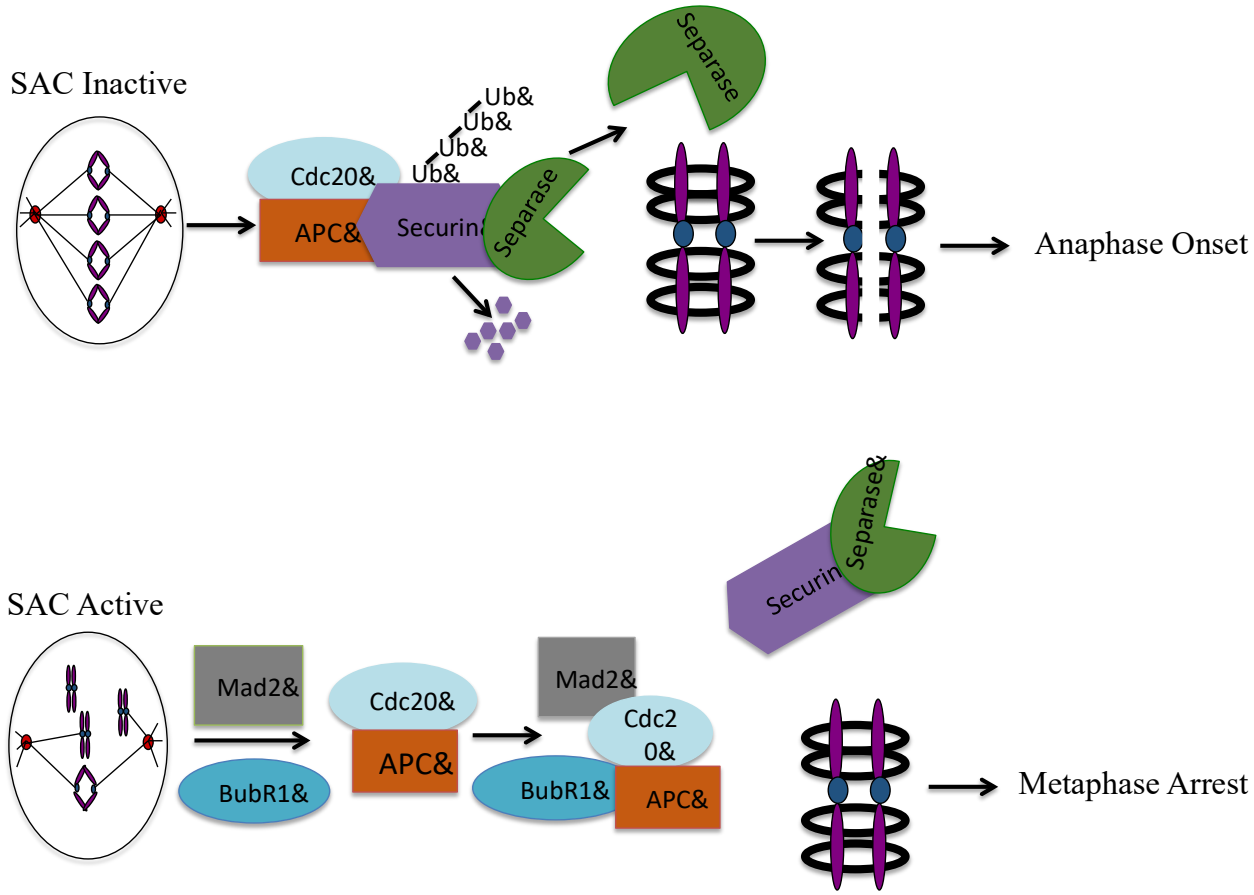

Figure 2. *Spindle Assembly Checkpoint (SAC) activation*
When each and every chromosome establishes bipolar attachments (upper panel), Cdc20 targets APC for securin degradation. Degradation of securin releases the inhibition on separase. Active separase, cleaves cohesin between sister kinetochores, thus chromosomes segregate and the cell proceeds to anaphase. In the presence of improperly attached kinetochores, SAC gets activated (bottom panel). Mad2 and BubR1 bind to Cdc20, forming the mitotic checkpoint complex, which inhibits the APC activity. As a result, securin does not get degraded and separase remains inactive. Therefore, chromosomes cannot be separated and the cell remains in metaphase (mitotic arrest).

Although several types of cancer cell lines display partial or complete loss of SAC activity, many aneuploid cell lines and human cancers do not contain mutations in SAC genes. One possible explanation for this phenomenon is that SAC defects in these cells are due to mutations in yet unidentified SAC genes. Another possibility is that instead of a complete loss of SAC activity, which leads to too frequent mistakes in chromosome segregation leading to cell lethality, weakened SAC activity is the primary driving force in tumorigenesis (25). Consistent with this, SAC genes have been shown to be required for embryonic viability in mice and flies: knocking down Mad2 in mice results in embryonic lethality (7), deleting Bub1 causes embryonic lethality in Drosophila (6). Data from different groups demonstrated that the SAC genes are essential for viability in vertebrates as well (26).

Targeting the SAC Against Cancer

Cancer cells are characterized by uncontrolled growth. Therefore, it is not surprising that drugs used to disrupt mitotic progression, referred to as “anti-mitotics”, are very effective in cancer therapy (27). Since mitosis is considered the most vulnerable phase of the cell cycle to various external factors such as exposure to radiation (28) and chemicals (29), arresting cells in mitosis (increasing the time they spend in mitosis) is favorable for cancer therapy.

MTs are one of the most successful targets in cancer chemotherapy (30). Vinca alkaloid (vincristine, vinblastine) and taxane group (paclitaxel/taxol, docetaxel) anti-mitotic drugs, which are successfully used in the treatments of several types of solid tumors as well as haematological malignancies (31), target MTs to induce a mitotic arrest in cancer cells. Taxanes, stabilize MTs by interfering with MT dynamics (27). KTs of taxol-treated cells are able to bind taxol-stabilized MTs, however, tension cannot be generated between sister KTs due to lack of MT dynamics (32). Vinca alkaloids, on the other hand, prevent MT polymerization by binding to β-tubulin at the plus ends of MTs (33): at higher concentrations, they induce MT depolymerization (27). Therefore, KTs of vinca alkaloid-treated cells cannot bind to MTs. Although the specific effects of the two groups of anti-mitotic drugs on MTs are different, they both prevent proper KT-MT attachments, which in turn activate the SAC leading to mitotic arrest. Exposure of every cell line tested until today to anti-mitotic drugs resulted in prolonged mitotic arrest through chronic SAC activation (34). SAC activated cells enter into mitosis but fail to exit. Therefore, mitotic index increases significantly when the cells are treated with anti-mitotic drugs (35).

Although MT-targeting anti-mitotic drugs are being used with great success in clinic today, resistance and toxicity are two factors limiting their clinical effectiveness. A major limitation in the use of MT targeting drugs is toxicity. Although cancer cells are more sensitive to MT-targeting drugs, division in normal cells is also affected by these drugs, because these drugs disrupt MTs in both cancer and normal cells. Disrupting MTs in rapidly dividing bone-marrow cells may lead to myelosuppression, which is a common side effect of vinca alkaloids chemotherapy (36). Myelosuppression is reversible, thus it is clinically manageable. Another type of toxicity caused by MT-targeting drugs is neuropathy. Neuropathies may occur due to MT disruption in non-dividing neurons or disruption of axonal flow by the formation of abnormal bundles of MTs. Unlike myelopathies, neuropathies are usually permanent, therefore more problematic in clinic (37).

Another limiting factor for the effectiveness of MT-targeting drugs in clinic is resistance (38, 39), which is commonly observed especially in taxol chemotherapy. Resistance to MT-targeting drugs may develop due to mutations in the tubulin subunits leading to alterations in drug binding, as reported for paclitaxel-resistant human ovarian cancer cells (40). Alterations in tubulin subunit expression may also lead to resistance. Overexpression of βIII isotype of tubulin has been shown to be associated with resistance to taxanes both in vitro (41) and in vivo (42).

There has been a significant effort to develop novel anti-mitotic drugs, which do not directly interfere with MT structure/dynamics, to overcome toxicity and resistance seen with MT-targeting drugs (43). New generation anti-mitotic drugs involve agents targeting mitotic kinases (Aurora B, Cdk1, Plk1 etc.), which are essential regulators of mitotic progression and motor proteins (CENP-E, Eg5 etc.), required for chromosome segregation. However, despite the promising preclinical data, next generation anti-mitotic drugs failed in clinical trials so far with moderate to severe side effects (43).

Diversity in Cellular Responses to Anti-mitotic Drug-induced SAC Activation

Chemotherapeutic SAC activation resulted in a prolonged mitotic arrest in all cancer cells tested so far (34). However, fate of the cells following the prolonged arrest is highly variable (Figure 3). It has been demonstrated that cells from different cell lines, even genetically identical cells of the same cell line, may respond to the

prolonged arrest in different ways (44). A better understanding of the molecular basis of this variation may provide helpful information to explain the clinical variation observed in response to cancer treatment, thus may lead to development of better strategies for cancer treatment.

One of the possible outcomes following the prolonged mitotic arrest is apoptotic cell death in mitosis. Apoptosis, or "programmed cell death", is a tightly regulated and complex cell suicide program involving multiple signaling pathways. Different apoptotic pathways can be triggered by different intra- and extracellular signals including DNA damage, genetic instability, oxidative stress and activation of the pro-apoptotic receptors on the cell membrane. Different apoptotic pathways merge at the final stage of apoptosis involving caspase activation, membrane blebbing and DNA fragmentation (31). Apoptosis has been considered as a major mechanism through which anti-mitotic drugs kill cancer cells (14). However, the molecular mechanism linking prolonged arrest to apoptosis is currently unknown. A recently identified apoptotic pathway involving Mcl1 has been reported to couple prolonged mitotic arrest to cell death (45). Mcl1 is an anti-apoptotic member of the apoptosis regulator Blc2 family. Mcl1 prevents apoptosis by inhibiting Bak and Bax association with mitochondria, which would otherwise generate pores in the mitochondrial membrane to induce cytochrome c release and initiate apoptosis. Therefore, an increase in the Mcl1 expression level is associated with cell survival, whereas a decrease in its expression is related to cell death. Anti-mitotic drugs, such as colchicine, have been shown to cause an increase in Mcl1 expression. Increased Mcl1 levels may provide the cell with the time to correct errors or make important cell fate decisions such as staying viable or committing to apoptosis (46).

Another possible cell fate following anti-mitotic drug-induced prolonged mitotic arrest is "adaptation". Mitotic exit in an unperturbed mitosis requires inactivation of the Cdk1-cyclin complex via cyclin B degradation (47). Once each and every chromosome establishes bipolar attachments, SAC gets satisfied and cyclin B is degraded. In the presence of improper attachments, SAC activity prevents cyclin B degradation, thus mitotic exit (2). It has been demonstrated that in anti-mitotic drug treated cells, cyclin B is slowly degraded despite the chronic SAC activation (44). Slow cyclin B degradation eventually falls below a threshold that allows the cell to exit from mitosis without cell division. This process is known as "adaptation".

In the presence of anti-mitotic drugs cells cannot achieve proper attachments, leading to chronic SAC activation. Under these conditions Mcl1 and cyclin B degradation rates are crucial to determine the cell fate. If Mcl1 levels drop before cyclin B levels fall below the threshold, cells initiate the apoptotic pathway before they exit mitosis (mitotic death). However, if cyclin B levels fall below the threshold for mitotic exit before Mcl1 levels decline, cells exit mitosis (adaptation) (46). Adapted cells exit mitosis with a 4N DNA and they either 1) die in G1 by apoptosis, 2) arrest in G1 (senescence), or 3) proceed to the next cell cycle (34, 48) (Figure 3).

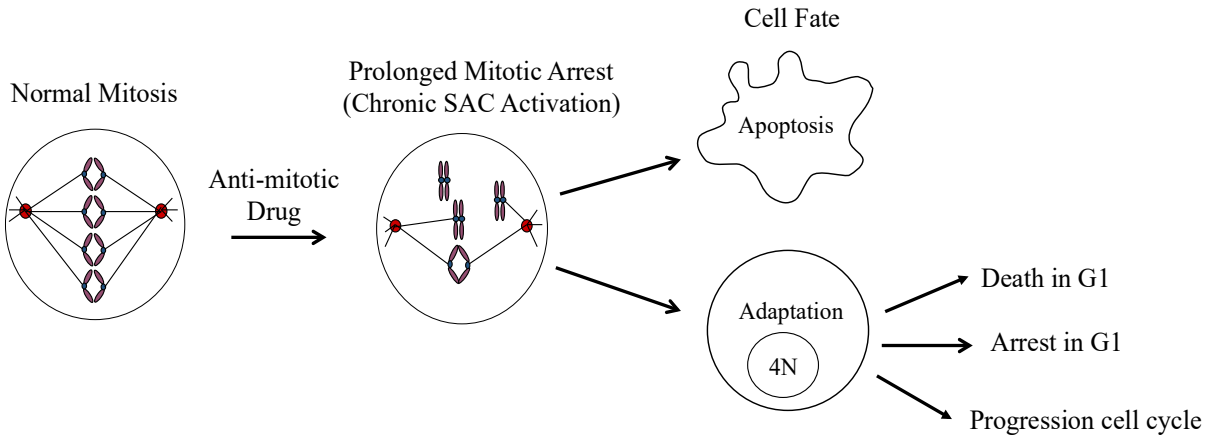

Figure 3. *Different cell fates in response to anti-mitotic drugs*
Anti-mitotic drugs lead to a prolonged mitotic arrest in cancer cells due to chronic spindle assembly checkpoint activation. Arrested cells can follow different post-mitotic fates following the prolonged arrest. They can either die in mitosis by apoptosis or undergo adaptation by exiting mitosis without cell division. Adapted cells may then either die in the next G1, arrest in the next G1 or progress into a new cell cycle.

Conclusions

Anti-mitotic agents are an important part of cancer therapy. Two groups of anti-mitotic drugs successfully used in chemotherapy, taxanes and vinca alkaloids, prevent proper KT-MT attachments by targeting MT dynamics/polymerization, and thus activate the SAC. Chemotherapeutic SAC activation leads to a prolonged mitotic arrest in cancer cells. Different cancer cells may respond differently to the prolonged arrest. One of the possible responses, apoptosis, is a major mechanism by which anti-mitotic drugs kill cancer cells. On the other hand, another possible response to the prolonged arrest, adaptation, enables cancer cells to survive by escaping from mitosis and proceeding to the next cell cycle despite the unsatisfied SAC. Adaptation may be a possible explanation for resistance, which is a major factor limiting the activities of anti-mitotic drugs in clinic. Therefore, controlling the cell fate in response to the prolonged mitotic arrest and driving the arrested cells toward apoptosis would significantly increase the efficacy of the anti-mitotic drugs. Influencing the threshold levels of Mcl1 and cyclin B is a possible way to induce cell death: Enhancing the effects of these drugs to better stabilize cyclin B would maintain the cells in mitosis, providing them enough time to degrade Mcl1 to induce apoptosis. On the other hand, triggering an increased Mcl1 degradation would also induce an early apoptosis. A better strategy in cancer treatment would be both inducing a prolonged arrest in mitosis and driving the arrested cells toward and apoptotic fate.

Developing new agents against mitotic specific targets, which disrupt mitosis but do not affect non-dividing cells, might also help eliminate the neurological side effects of the anti-mitotic drugs used in clinic today. However, mitosis specific agents - targeting mitosis specific kinases and motor proteins that are required for chromosome segregation in mitosis - that have been developed until today failed in clinical trials.

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