REVIEW ARTICLE

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Unraveling pathologies underlying chromosomal instability in cancers

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Abstract

Aneuploidy is a widespread feature of malignant tumors that arises through persistent chromosome mis-segregation in mitosis associated with a pathological condition called chromosomal instability, or CIN. Since CIN is known to have a causal relationship with poor prognosis accompanying by multi-drug resistance, tumor relapse, and metastasis, many research groups have endeavored to understand the mechanisms underlying CIN. In this review, we overview possible etiologies of CIN. The key processes to achieve faithful chromosome segregation include the regulation of sister chromatid cohesion, kinetochore-microtubule attachment, bipolar spindle formation, spindle-assembly checkpoint, and the activity of separase. Aberrant chromosome structures during DNA replication might also be a potential cause of CIN. Defective regulation in these processes can lead to chromosome mis-segregation, manifested by lagging chromosomes, and DNA bridges in anaphase, leading to gross chromosome rearrangements. Investigation into the molecular etiologies of CIN should allow us to explore novel strategies to intervene in CIN to control cancers.

KEYWORDS
Aurora B, chromosomes, kinetochores, microtubules, separase, SMC complex

1 | INTRODUCTION

The human body is composed of *c.* 60 trillion cells. The homeostasis of organs and tissues is maintained based on a proper control of cell proliferation and the underlying cell cycle. In cancer cells, however, the cell cycle is mostly out-of-control: the "accelerators" and "brakes" of cell cycle are impaired. Importantly, this condition is often accompanied by defective cell division and chromosome mis-segregation. This condition called chromosomal instability, or CIN, and generates cells having an aberrant number of chromosomes deviating from the diploid number, 46, and results in a massive tumor formation consisting of cells having different number and structure of chromosomes, ie, aneuploidy.[1] Additionally, chromosome mis-segregation in mitosis can originate from defects outside of mitosis, including DNA replication. In this review, we will overview the mechanism for this cellular pathology, based on our latest knowledge on chromosome segregation control.

2 | CONTROL OF KINETOCHORE-MICROTUBULE ATTACHMENT

In early mitosis, microtubules are assembled into mitotic spindles in which split centrosomes serve as microtubule organization center at both poles. A faithful chromosome segregation requires that each pair of sister kinetochores binds to microtubules emanating from opposite poles so that sister chromatids move into daughter cells following the release of sister chromatid cohesion. Because microtubule attachment at kinetochores proceeds only stochastically, cells inevitably create error-prone attachments, such as merotelic attachments (a kinetochore attaches to microtubules from both direction), at some incidence.[2] To maintain the ploidy, cells have the ability to correct these erroneous attachments, which depends on both dynamic nature of microtubule attachments and activity of the mitotic kinase Aurora B (Figure 1).

The former, dynamic microtubule-dependent mechanisms have been identified by the series of pioneering works by Duane Compton and his colleagues.[3–6] By measuring tubulin turnover rate (using photoactivatable tubulin), the time to exchange the half of tubulin (of kinetochore-attached microtubules) was found to be physiologically controlled in a narrow window, ie, 2-3 min in prometaphase and 4-5 min in metaphase. Extension of a few minutes of these times was enough to already induce chromosome mis-segregation in cancers, suggesting that microtubule attachments must be sufficiently dynamic to permit correction of erroneous attachments.

In the latter, Aurora B kinase plays a central role in the correction mechanism by directly phosphorylating kinetochore proteins such as Hec1/Ndc80 and destabilizes microtubule attachments.[7,8] How Aurora B enriched at inner centromeres targets kinetochore proteins specifically on those with erroneous attachments remains enigmatic, but spatial distance of kinetochores from the centromere seems to be a decisive factor: kinetochores that had established correct attachments will be under a pulling force from the opposite pole and the activity of Aurora B does not reach to the distantly positioned kinetochore substrates. By contrast, kinetochores with merotelic attachments are pulled from both poles and therefore stay in close proximity to inner centromeres. Kinetochore proteins are then susceptible to be phosphorylated by Aurora B, which makes microtubule attachments unstable[9,10] (Figure 1). Then, the phosphorylated kinetochore proteins are dephosphorylated by phosphatases such as PP1 and PP2A to regain abilities to bind anew with microtubules.[11,12] Through the release of erroneous attachments one after another, kinetochores eventually attain proper attachments in full. These established attachments promote PP1 recruitment to kinetochores and suppress Aurora B activity, enhancing the stability of microtubule attachments.[13,14] Unlike other erroneous attachment types, merotelic attachment is not sensed by the spindle-assembly checkpoint and therefore its correction to prevent chromosome mis-segregation relies significantly on the correction mechanism.[15]

We found that a heterochromatin protein called HP1 is an essential member of the Chromosomal Passenger Complex (CPC) consisting of Aurora B, Borealin, Survivin, and INCENP and it is required to obtain high activity of Aurora B[16] (Figure 2A). The contribution of HP1 becomes particularly relevant when Aurora B phosphorylates kinetochore substrates and destabilizes erroneous microtubule attachments. Of note, the amount of HP1 associated with the CPC is widely reduced in cancer cells which consistently causes reduced output of Aurora B activity and increased rate of lagging chromosomes.[16] As centromeric kinesins regulating microtubule dynamics such as MCAK and Kif2B[5] are Aurora B substrates, to what extent the decline of Aurora B activity might contribute to the over-stabilizing kinetochore microtubules is an interesting question.

Enrichment of Aurora B at centromeres is known to be mediated by histone phosphorylations, by Bub1 and Haspin kinases. The Bub1-mediated phosphorylation of H2A at Thr120 recruits Sgo1 and thereby CPC through Borealin.[17] Phosphorylated histone H3 Thr3 (H3T3ph) by Haspin kinase interacts with CPC through Survivin and contributes to centromeric localization of Aurora B.[18]
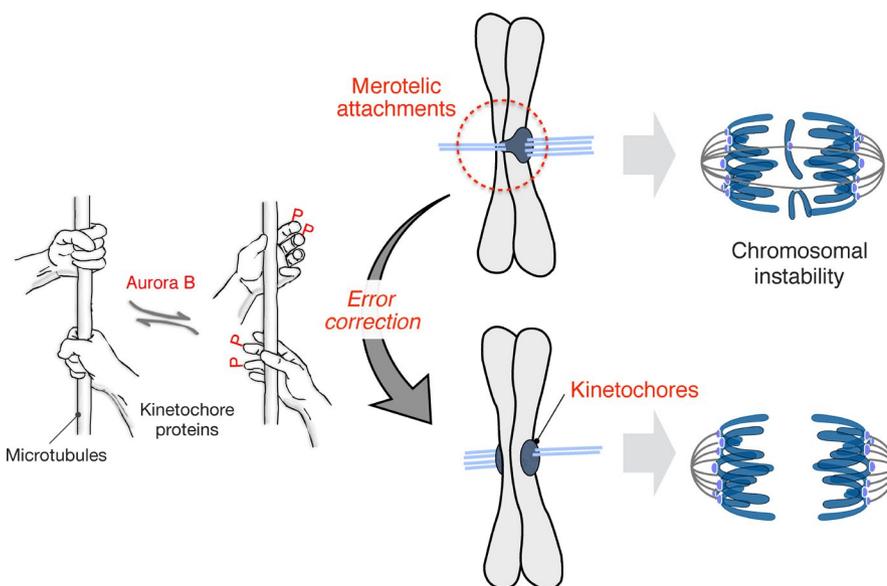

FIGURE 1 The error correction mechanism destabilizes erroneous microtubule attachments such that all the chromosomes become bioriented on the mitotic spindle. Central to this mechanism is the dynamics of microtubules and the activity of Aurora B, which phosphorylates kinetochore proteins and destabilizes the microtubule bindings as illustrated
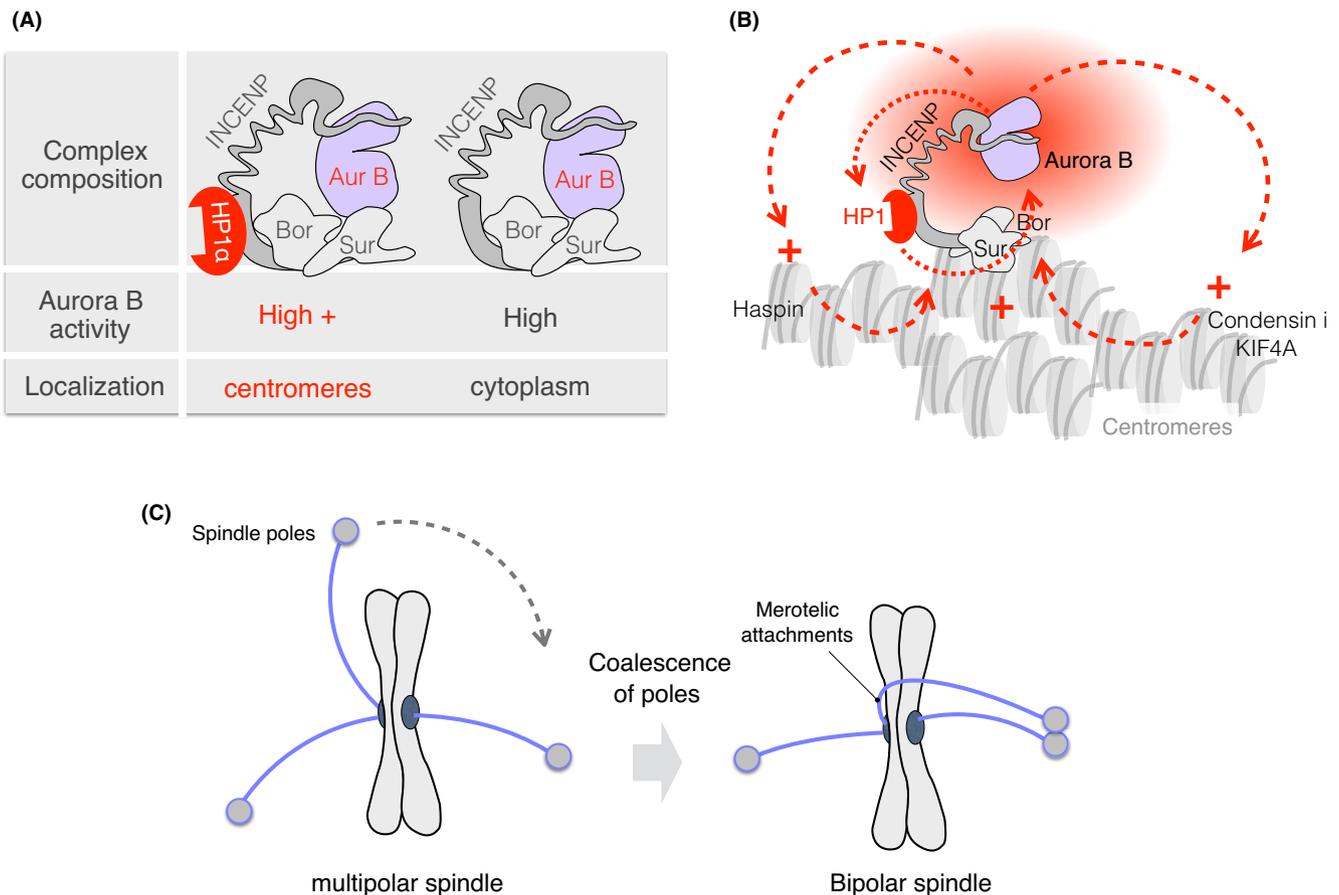
(A)

Complex composition	
Aurora B activity	High + High
Localization	centromeres cytoplasm

(B)

(C)

FIGURE 2 Regulation of Aurora B activity at centromeres. A, Aurora B is the catalytic subunit of the protein complex called the Chromosomal Passenger Complex (CPC), with INCENP, Borealin (Bor) and Survivin (Sur). HP1 is the fifth subunit of this complex providing higher kinase activity to Aurora B, and HP1-containing CPC is enriched at centromeres. B, System level control of Aurora B kinase, which is supported by a number of positive feedback circuits (see text for details). C, Supernumerary centrosomes increase the prevalence of merotelic attachments. In cells with extra number of centrosomes, multipolar spindle is often formed, and kinetochore-microtubule attachment can be merotelic during coalescence of multi- to bipolar spindle. Likewise, any conditions that disrupt spindle geometry, eg, nocodazole release, generate merotelic attachments with higher incidence

The activity of Aurora B seems to be maintained at centromeres through additional layers of feedback mechanism[19] (Figure 2B). As mentioned above, the kinase activity of Aurora B is enhanced by HP1, and the binding of HP1 becomes stable when HP1 is being phosphorylated by Aurora B.[16] Moreover, Aurora B phosphorylates condensin I, and condensin I is required for chromatin compaction and confers physical rigidity to centromeres.[20,21] This structural property is in turn required to concentrate Aurora B activity at centromeres. Therefore, reduced Aurora B activity in cancer cells can also be indicated by unfocused and diffusible centromeric localization of Aurora B.[22]

Notably, Aurora A kinase, another class of Aurora kinase, has also been implicated in correcting the error-prone kinetochore-microtubule attachments. Unlike Aurora B, however, Aurora A destabilizes attachments by phosphorylating Hec1/Ndc80 when kinetochores become closed to spindle poles during chromosome oscillation movements.[23–25]

3 | INCREASED NUMBER OF CENTROSOMES

Centrosomes replicate under the activity of cyclin E-cdk2 kinase in early S-phase and are distributed to daughter cells in M phase. In addition, centrosome replication is controlled so that it occurs only once per cell cycle, similar to that of chromosomes. However, the number of centrosomes is often increased due to over-replication of centrosomes in cancer cells, due to over-action of cyclin E/cdk2 and of Polo-like kinase-4, or Plk4, and loss or inactivation of p53. Defective p53 function may give rise to cytokinesis failure, in which case duplicated centrosomes will be carried over in the undivided cell.[26] As a result, mitotic spindle becomes multipolar. Although these spindle poles in many cases coalesce into 2 and form a bipolar spindle, multipolar configuration provides high chance of forming merotelic attachments[27,28] (Figure 2C). Therefore, increased number of centrosomes is causally related to CIN.

4 | CONTROL OF THE SPINDLE-ASSEMBLY CHECKPOINT

The simultaneous and synchronous separation of sister chromatids in anaphase is a symbolic process of cell division. The ubiquitin-ligase APC (anaphase-promoting complex) is the key enzyme that initiates the whole program of anaphase, by inducing separase activation and Cdk1 inactivation.[29] The spindle-assembly checkpoint, or SAC, is a negative feedback signaling mechanism that suppresses the function of APC.[30-32] It literally works when the mitotic spindle is not properly assembled, but this signaling remains active unless all the kinetochores in the cell become occupied by microtubules. In unperturbed mitoses, switching off the SAC and the inverse activation of the APC proceed during metaphase[33] to determine an appropriate timing to induce chromosome separation in anaphase. When SAC does not operate properly, due to a defective sensing or signaling system, cells fail to secure enough time for biorientation of sister chromatids and to equally transmit the sister chromatids.

Mosaic variegated aneuploidy (MVA) syndrome is a disease caused by a germline deficiency or mutation in the gene of a SAC component BubR1, and characterized by growth retardation, microcephaly, and the development of childhood cancers.[34] In MVA cells, the function of SAC is impaired and therefore results in the accumulation of aneuploid cells, which is thought to increase the risk of carcinogenesis.[35] Somewhat unexpectedly, somatic mutations in SAC genes are not generally found in cancers. Several lines of experiment have shown that the quantitative control of the SAC components is important: halving the expression level of Mad2 or Mad1 is shown to cause defective SAC function, ie, haploinsufficiency, and, conversely, increased levels of Mad1 and Mad2 seem to impair a proper checkpoint response.[36-38] Despite these observations, it is worth noting that cancer cell lines can respond to spindle-assembly defects and are in principle proficient for the SAC function.[6] Therefore, to what extent SAC impairment might contribute to CIN in clinical cases seems to be unclear.

The signaling of SAC is catalyzed at kinetochores that are not yet attached to microtubules. Therefore, the ability of correction mechanisms to dissociate erroneous microtubule attachment and to generate unattached kinetochores is crucial to ensure extra time to prevent chromosome mis-segregation. In this context, the reduced ability of correction mechanisms, eg, insufficiency of Aurora B function, may appear as if SAC function is weakened. To differentiate a defect in correction machinery or in the SAC system per se is important in searching the causes of CIN (Figure 3).[39]

What tips the switch of SAC from ON to OFF state in response to microtubule attachments? Phosphorylation and dephosphorylation of a kinetochore protein called Knl1 is an instrumental piece comprising this switch (Figure 4A).[40] In prometaphase, Mps1 kinase is recruited to unattached kinetochores, and it phosphorylates the MELT motif of Knl1. The phosphorylated MELT motif serves as a platform for SAC proteins including Bub1, BubR1 and Mad1/Mad2. This promotes the formation of a mitotic checkpoint complex, MCC, consisting of Mad2, BubR1, Bub3, and Cdc20 (Figure 4B), which
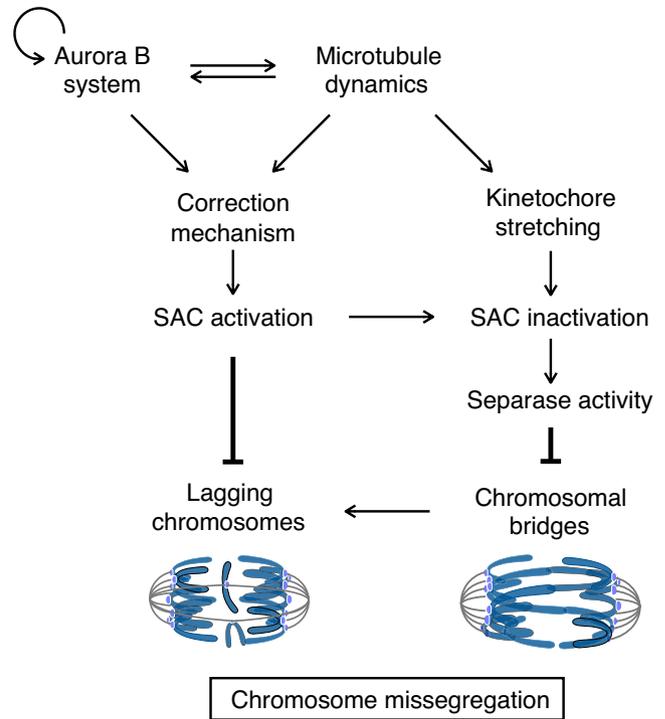

FIGURE 3 Cellular functions preventing chromosomal instability in mitosis. There seems to be at least 2 pathways that lead cells to chromosome mis-segregation: defective correction of erroneous microtubule attachments causes lagging chromosomes and defective SAC inactivation and subsequent separase activation cause chromosomal bridges, which may account for numerical and structural chromosomal instability, respectively (see text for details). Proficient Aurora B activity and microtubule dynamics are essential for these mitotic machineries

directly binds and inhibits APC.[41] Upon microtubule attachment, Mps1 becomes delocalized from kinetochores.[42] This proceeds efficiently because Mps1 and microtubules share a binding site on Hec1 at kinetochores.[43] In addition to Mps1 departure, Knl1 recruits phosphatase PP1 to promote reversion of MELT motif phosphorylation and release of the SAC components.[42]

To understand how mechanistically Knl1 recruits PP1 is a challenging question, but it is possible that "kinetochore stretching" may contribute to this process (Figure 4B): After kinetochores become attached to microtubules on its ends, the distance between the inner and outer layers of kinetochore continuously changes by virtue of microtubule growth and shrinkage. This motion called kinetochore stretching occurs because the side of microtubule tips is held by the outer layer, which is linked to the inner layer by the long flexible region of CENP-T. Perturbation of kinetochore stretching indicates its involvement in SAC inactivation.[44-46] Interestingly, Mps1's delocalization from kinetochores takes place irrespectively of stretching, but it seems to involve the removal of Mad1 and BubR1. An implication from these observations is that SAC inactivation proceeds in two steps: first by Mps1 eviction and second by kinetochore stretching. What seems to be relevant to CIN phenotype is that
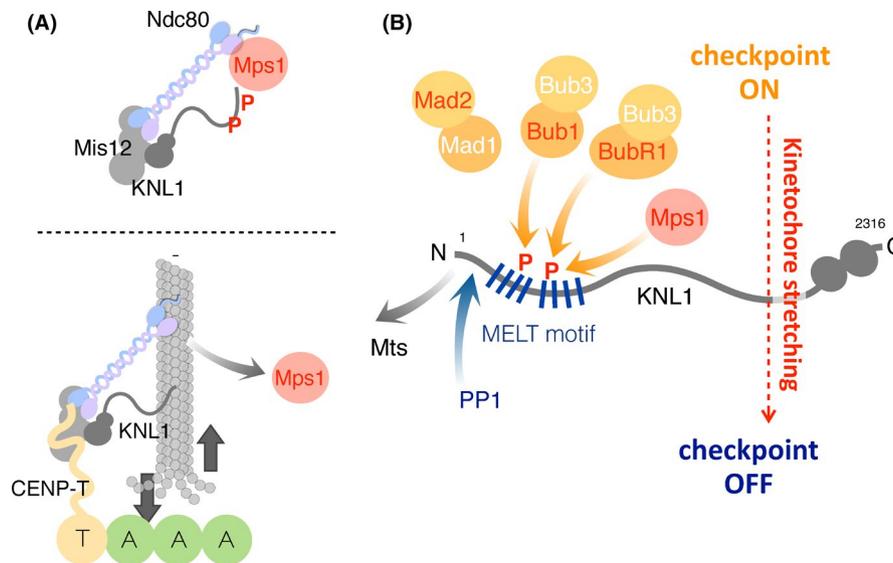
FIGURE 4 Switching on and off of the SAC signal at kinetochores. A, The network with 3 protein complexes, or KMN network, including the Ndc80 complex, the Mis12 complex, and the Knl1 complex, comprise the microtubule capture module at outer plate of kinetochores. At unattached kinetochores Mps1 kinase is bound to Ndc80 (upper scheme). Upon microtubule attachment, Mps1 is removed and kinetochores experience stretching, as microtubules push kinetochore interior (A: CENP-A nucleosome) back and forth (lower scheme). B, When SAC is active, Mps1 phosphorylates MELT motif of Knl1 and that recruits SAC proteins at kinetochores. After microtubule attachment and Mps1 departure, kinetochore undergo stretching and recruits PP1 which promote SAC inactivation by releasing SAC proteins from kinetochores. Association of N-terminal of Knl1 to microtubules (Mts) has been implicated in this process

many cancer cells reveal reduced incidence of kinetochore stretching, which should contribute to a delay in metaphase.[46]

5 | DISSOCIATION OF SISTER CHROMATID COHESION AND REGULATION OF SEPARASE

In order for cells to accomplish equal transmission of the genome to the next, replicated DNA molecules are assembled to package them into sister chromatids. These sister chromatids are paired by cohesion, in which a ring-shaped protein complex called cohesin holds replicated DNA molecules. Removal of cohesin is therefore required to separate sister chromatids, and cohesin is known to be removed in two processes during mitosis: first, by the end of metaphase, cohesin on chromosome arms is dissociated by phosphorylation of the cohesin SA subunit, whose reaction is mediated by Plk1 (Polo-like kinase 1). This process, called the prophase pathway, unloads most of the cohesin complexes from the arms, but leaves them at centromeres.[47] Then, in anaphase, the protease separase becomes active and cleaves the cohesin subunit Scc1/Rad21 of the centromeric cohesin.[48] Upon removal of all the cohesin from chromosomes, sister chromatids will be separated at the onset of anaphase.

The timely removal of cohesin at the metaphase-to-anaphase transition is required to prevent chromosomal mis-segregation, and control of separase is key to this process.[48] A probe for separase activity characterized the activation profile during metaphase-to-anaphase transition: separase is kept inactive through much of metaphase until the time when its abrupt activation occurs, shortly

before the anaphase onset [49] (Figure 5A). Importantly, separase activity was detected only when the probe was engineered to be place in the chromosomes, but did not when placed in the cytoplasm. This seems to ensure that separase will work properly in the vicinity of chromosomes irrespective of the cell size, including eggs with a large cytoplasm and cancer cells with a small volume of cytoplasm.[50] In addition, immediately after separase removes cohesin as a protease, it turns into a suppressor of Cdk1 and promote the poleward movement of sister chromatids to the mitotic spindle pole.[49,51] By coordinating two anaphase events, separase plays a key role to ensure chromosome segregation.

Therefore, defective separase control must relate to CIN, but how separase regulation is altered in cancer cells has remain uncharacterized. Using separase to probe these cancer cells, we found that separase becomes active well before anaphase onset, and with this precocious activation (precocious with regard to anaphase onset).[52] As mentioned before, cancer cell lines often reveal various extents of metaphase delay, and separase becomes active during this extended period of metaphase. Remarkably, following precocious activation, separase attains lower levels of protease activity at anaphase onset. This alteration in separase activation profile therefore leads to DNA bridges in anaphase due to incomplete removal of cohesin.[52] Altogether, it seems that cancer cells are often associated with a problem in metaphase-to-anaphase transition: a delayed inactivation of SAC slows down metaphase progression, and this perturbs the activation kinetics and enzymatic proficiency of separase (Figure 5B). The resulting anaphase bridges must contribute to CIN, either by structural instability induced by DNA breakage or by ploidy changes through non-disjunction of sister chromatids.
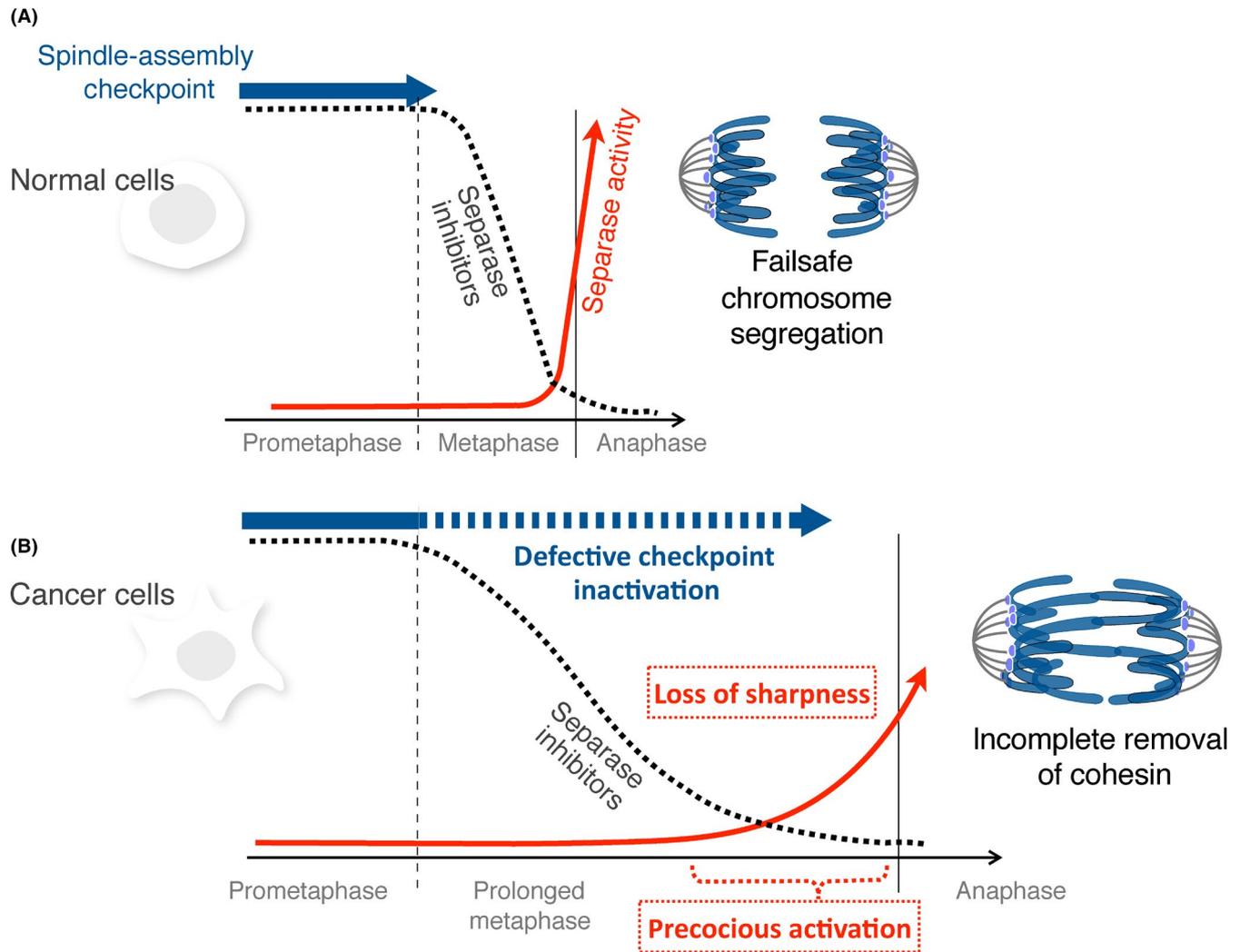
FIGURE 5 Regulation of the metaphase-to-anaphase transition. A, In non-transformed, chromosomally stable cells, SAC inactivation, and APC activation proceed swiftly, which is followed by an abrupt and sharp activation of separase. B, In many cancer cell lines, SAC inactivation, and APC activation proceed slowly and therefore metaphase is extended. During prolonged metaphase, separase become precociously active but fail to activate in full at anaphase onset. This causes incomplete removal of cohesin and prominent chromosomal bridges in anaphase

6 | INVOLVEMENT OF DNA REPLICATION

Defective regulation of M phase events is considered to be primarily related to CIN acquisition, but it should be noted that the DNA replication process is also inherent to stable chromosome distribution. Any obstacles to replication fork progression can perturb this process: for example, chemicals and proteins that bind to DNA, DNA breaks associated with DNA damage responses, twisted structures of DNA that accumulate between replication forks, or gigantic transcription devices. The presence of these structural blockades could significantly delay DNA replication and could be a source of gross chromosome rearrangements.[53]

Because cells seem to lack a robust function to monitor the completion of replication, cells can enter M phase without completing DNA replication, and in such cases, DNA synthesis continues during mitosis. This phenomenon called MiDAS (mitotic DNA synthesis) is typically seen in DNA domains undergoing late replication

timing, including pericentromeric heterochromatin or subtelomeres, and implicated in expression of common fragile sites.[54,55] Despite MiDAS, unreplicated DNAs in mitosis will be "unresolved structures" between sister chromatids, and chromosome separation in its presence results in bridged DNA in anaphase. They may generate thinner structures called ultra-fine DNA bridges (UFBs) which are not stained (not intercalated) by DNA dyes but decorated with proteins involved in repair, such as BLM, PICH, and FANCD2.[53,56] Whereas normal human cells undergoing anaphase can form UFBs, significant replication delay can cause bulky chromosome bridges that DNA dyes can intercalate. Although these replication-related chromosome bridges are different from those generated by fusion of broken chromosome ends, known as the classical breakage/fusion/bridge cycle,[57,58] chromosome bridges in general are hardly lacerated during anaphase and telophase and thereby culminate in lagging chromosomes, undergoing unbalanced segregation (Figure 3). Furthermore, long-lasting chromosome bridges may interfere with

FIGURE 6 Replication forks may encounter various obstacles arisen on DNA. For instance, unwinding parental DNA strands generate overwound supercoils ahead of forks and catenated sister chromatids behind. Unless these supercoils are resolved during and after replication, replication is uncompleted and nodes of entangling sister chromatids remain until the beginning of anaphase. In cells lacking SMC5/6, these supercoils are not efficiently resolved
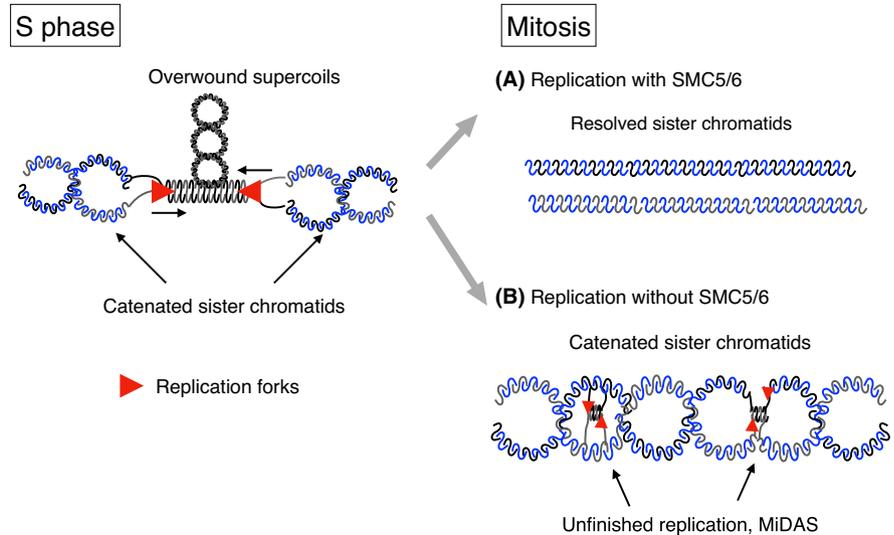
cytokinesis. The resulting tetraploid cells with whole-genome duplication is known to become more susceptible to generating aneuploid progeny.[59]

The SMC5/6 complex, a member of SMC family consisting of cohesion and condensin, has been implicated in DNA replication and repair,[60] and depletion of this causes extensive DNA bridges in anaphase.[61] SMC5/6 seems to be required to simplify the topological entanglements and supercoil structures generated during DNA replication, which impede progression of replication forks unless removed (Figure 6, Y. Kusano & T. Hirota, unpublished data). Although the incidence of mutations in each SMC5/6 subunit in cancer is rarely observed, according to COSMIC database, a possibility of pathological relevance of SMC5/6 dysfunction in cancer seems worth noting; because cells with dysfunctional SMC5/6 are prone to form aneuploid chromosomes.

It is also known that the failure of mitotic chromosome segregation leads to the disruption of the replication process in the next S-phase, which may give rise to multiple gross rearrangements on whole chromosome length in a phenomenon called chromothripsis.[62] Lagging chromosomes sometimes form micronuclei independently of the original nucleus, and shortage of the factors required for DNA replication in these micronuclei is thought to break chromosomes into pieces and re-connect disorderly.[63] In these chaotic chromosomes, the genome sequence is disrupted and fused, which may lead to activation of oncogenes, inactivation of tumor suppressor genes, or development of specific fusion genes with carcinogenic activity.

7 | CONCLUSION

There are multiple reasons that can lead cells to chromosomal instability, and causal relationship between errors in controlling mitotic chromosome dynamics has now become into focus. Live cell imaging analysis has importantly pointed out that, in aneuploid cancer cell lines, the incidence of chromosome mis-segregation is as low as one

chromosome nondisjunction in 1 to 5 divisions.[3] This may imply that larger magnitude of chromosomal mis-segregation will put cancer cells under harsh and toxic chromosomal stress beyond the tolerable level that allows neither survival nor proliferation. It is indeed a relatively small-scaled chromosomal mis-segregation that associates with the development and progression of cancers. Understanding these pathological ranges is essential to further identify the cellular pathway to chromosomal instability as well as to explore strategies to intervene in CIN to control cancers.[64]

DISCLOSURE

The authors have no conflict of interest.

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