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DNA damage associated with mitosis and cytokinesis failure

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Abstract

Mitosis is a highly dynamic process, aimed at separating identical copies of genomic material into two daughter cells. A failure of the mitotic process generates cells that carry abnormal chromosome numbers. Such cells are predisposed to become tumorigenic upon continuous cell division and thus need to be removed from the population to avoid cancer formation. Cells that fail in mitotic progression indeed activate cell death or cell cycle arrest pathways, however, these mechanisms are not well understood. Growing evidence suggest that the formation of *de novo* DNA damage during and after mitotic failure is one of the causal factors that initiate those pathways. Here, we analyze several distinct malfunctions during mitosis and cytokinesis that lead to *de novo* DNA damage generation.

Keywords

Mitosis; DNA damage; telomeres; checkpoints; cytokinesis

Introduction

Mitotic progression is associated with a highly dynamic change of chromosome morphology and movement, which is traditionally classified into 5 stages: prophase, prometaphase, metaphase, anaphase, and telophase. Although an alternative definition of mitotic progression based on the changes in cell-cycle regulators has been proposed (1), we here apply the traditional definition based on the morphological change of typical vertebrate cells, since it is more relevant to the focus of this review. Mitosis starts when condensed chromosomes become visible in the prophase nucleus. Nuclear envelope breakdown (NEBD) terminates prophase and initiates prometaphase, during which microtubules emanating from separated sister centrosomes find and attach to kinetochores, a huge protein-DNA structure formed at the centromere (2). To avoid premature separation of sister chromatids, the spindle assembly checkpoint (SAC), which monitors mitotic progression during this time, is active until metaphase. When all sister chromatid pairs are aligned on the spindle equator, with all sister kinetochores properly attached to the spindle, the SAC is satisfied. Consequently, the anaphase promoting complex (APC), a ubiquitin ligase that induces destruction of its critical substrates cyclin B and securin (3), is activated. Destruction of cyclin B inactivates CDK1, the activity, which inhibits reactions required for mitotic exit, while securin destruction activates its target protease, separase. Released and activated separase cleaves cohesin that holds sister chromatids together, which leads to the onset of anaphase. During anaphase sister chromatids split away from each other by the force of microtubules and start decondensation when telophase begins. Then the nuclear envelope is reformed around the sets of daughter chromosomes during telophase, which is

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followed by cytokinesis, a distinct process that pinches off the separated daughter chromosomes via the cleavage furrow.

Failures during the mitotic process or cytokinesis potentially cause the formation of cells with abnormal ploidy, such as aneuploidy or tetraploidy. Tetraploid cells generated through cytokinesis failure are relatively unstable compared to their diploid counterparts and frequently become aneuploid upon continued cell division (4). In a wild type genetic background, cells with abnormal ploidy rarely outgrow owing to mechanisms, by which cells that spend an abnormal time in mitosis are removed from the population. These mechanisms are considered to be tumor suppressive because tetraploid and aneuploid cells are potentially tumorigenic (5–7). Indeed, the outgrowth of tetraploid cells is inhibited by tumor suppressors, p53 and Rb, which are frequently inactivated in tumors carrying abnormal hyperploid karyotypes (8).

One of the mechanisms that is associated with some degree of mitotic arrest owing to a failure to proceed with prometaphase or metaphase, is known as mitotic catastrophe, which has at least three different consequences: cell death during mitosis, cell death after mitotic exit, and senescence after mitotic exit (9). In the latter cases, cells exit mitosis before execution of the cell death pathway by eventual satisfaction of the SAC, or by gradual degradation of cyclin B in the presence of an active SAC, which allows for anaphase, telophase, and cytokinesis to be omitted (10). Mitotic catastrophe has well been described as a cause of cell death after premature onset of mitosis with unrepaired DNA damage, which is associated with a defective G2/M checkpoint (11). Inhibition of mitotic progression prior to the metaphase-anaphase transition by drugs, such as microtubule drugs, also causes mitotic catastrophe (9, 12, 13).

The other type of mitotic failure, which causes generation of lagging chromosomes, chromosome bridges, and/or micronuclei formation, is the premature onset of anaphase without prolonged mitotic arrest. This has been shown to be associated with a disruption of the SAC pathway, which subsequently causes cell death, while cells that have escaped death become aneuploid (14). Those aneuploid cells generally show reduced fitness and are outcompeted by diploid cells (15). Thus, besides mitotic catastrophe, distinct mechanisms remove those cells that failed at later mitotic stages.

Cell cycle arrest or apoptotic cell death in interphase following these mitotic failures are mainly controlled by the tumor suppressor p53 (11, 16, 17), although p53-independent mechanism also exists, since p53-deficient cells execute cell death pathways including necrosis in the following G1 phase after exposure to mitotic drugs (18). The cell death pathway occurring during prolonged mitotic arrest is p53-independent, and the involvement of caspase has been indicated at least in some cell lines (18–20). On the other hand, a failure during cytokinesis following a normal mitotic process leads to binucleated daughter cells, which have the potential to enter the next round of the cell cycle even in a p53-positive background (21, 22). These cells, however, encounter a problem in the next round of mitosis due to a doubling of their centrosome number, which causes multipolar mitosis and a high frequency of mitotic failure (23, 24). Thus, cells that experienced cytokinesis failure subsequently suffer from mitotic failure in the following cell cycle and are likely to be removed from the cycling population.

The pathways that induce p53 dependent and independent cell death or senescence during and after mitotic failures are not fully understood and probably consist of several distinct cues. The existence of a p53-dependent “tetraploidy checkpoint”, by which an abnormal number of chromosomes or centrosomes are sensed through unknown mechanisms (25), has been challenged by several studies (5, 21, 22, 26–28). Recent work has prompted an

involvement of DNA damage as one of the causal factors (29), where several lines of evidence have indicated that cells that have failed mitotic progression eventually accumulate de novo DNA damage foci during and after mitotic failure.

It has been established that DNA damage (e. g. double stranded DNA breaks or the accumulation of single stranded DNA) activates the DNA damage response (DDR) pathway, where central kinases such as ATM or ATR amplify a signal to halt the cell cycle. Although the initial goal of the DDR is to repair the DNA insult and restart the cell cycle, excess amount of damage or irreparable damage eventually induce an irreversible cell cycle arrest known as senescence, or programmed cell death. Thus, induction of DNA damage during and after mitotic failure is an attractive candidate that initiates cell death or permanent cell cycle arrest. In a checkpoint-compromised background, however, chromosomal damage associated with mitotic failure can be propagated to the next cell cycle, leading to structural alteration of chromosomes, which is potentially tumorigenic. These facts underscore the importance to understand the causes and consequences of DNA damage during and after mitotic arrest. In this review, mainly focusing on mammalian systems, we will first describe the DDR pathway during mitosis, and then discuss de novo DNA damage arising from different types of malfunctions during mitosis and cytokinesis.

The DDR during mitosis

The canonical DDR pathway

A DNA double strand break (DSB) is a highly cytotoxic insult that is immediately detected by DDR ‘sensor’ proteins, such as the Mre11-Rad50-Nbs1 (MRN) complex and Ku70–Ku80 heterodimer, and upstream phosphatidylinositol-3-kinase-like kinases (PIKKs) including ATM and DNA-dependent protein kinases (DNA-PK), all being recruited to the DSB site (30). Single stranded DNA that accumulates during DNA replication failure is coated by RPA, which recruits another PIKK family member, ATR, through interaction with ATRIP (30). The recruited PIKKs phosphorylate their prime target, the histone H2A variant H2AX on Ser-139, also known as γ–H2AX. Foci of γ–H2AX are cytologically visible and considered a hallmark of DNA damage. In the case of DSB, γ–H2AX is recognized and bound by a ‘mediator’ protein, MDC1, that amplifies the DDR signal by recruiting more MRN-ATM to the damage site (31), while ATR is maintained and activated by TOPBP1 at the damage site in a 9-1-1 (RAD9-RAD1-HUS1) complex-dependent manner (32). Another layer of the signal cascade is ordered recruitment of ubiquitin ligases RNF8 and RNF168, which promotes retention of 53BP1 and BRCA1 at DNA damage sites (33). Sustained active ATR and ATM phosphorylate ‘signal transducers’ such as CHK1 and CHK2 kinases, respectively, which consequently leads to phosphorylation-dependent regulation of ‘effectors’ including the Cdc25 family proteins and p53 (34).

The consequence of DNA damage during mitosis

When cells encounter insults during the G1, S or G2 phase of the cell cycle, the canonical DDR pathway delays cell cycle progression, which is well known as DNA damage (G1/S and G2/M) checkpoint or DNA replication (intra-S) checkpoint. In contrast, once cells are committed to enter mitosis, they do not activate the full DDR pathway upon DNA damage (35). The point of the commitment has been proposed to be in late prophase, since irradiation-induced DNA damage in early prophase but not late prophase causes a reversion of cell cycle to interphase (36, 37). Beyond this point, irradiation of prometaphase and metaphase cells still generates γ– H2AX foci on condensed chromosomes, however, downstream accumulation of RNF8, RNF168, BRCA1 and 53BP1 is attenuated (38, 39). Also, mitotic cells are incompetent in activating CHK2 kinase even in the presence of active ATM (40, 41), which is due to Polo-like kinase (Plk1)-dependent inhibitory phosphorylation

of CHK2 (41). In contrast to the *S. cerevisiae* system, where DNA damage during mitosis directly inhibits mitotic progression through DNA damage checkpoints (42, 43), mammalian mitotic cells do not have a functional link between DNA damage checkpoints and the SAC (37). Indeed, cells that are exposed to ionizing radiation (IR) during prometaphase and metaphase proceed to anaphase without delay (44), resulting in the activation of a full DNA damage checkpoint in the following G1 phase of the cell cycle (40). Although extensive DNA damage induced by irradiation during late prophase and prometaphase has been shown to cause SAC-dependent metaphase arrest, the delay does not depend on an ATM-mediated checkpoint pathway, suggesting that DNA damage that perturbs kinetochore-microtubule structure indirectly induces SAC activation (45). Besides direct irradiation of mitotic cells, premature entry into mitosis with unrepaired DNA damage in a G2/M checkpoint deficient background also generates mitotic cells displaying DNA damage, which consequently triggers mitotic catastrophe (13). Thus, dependent on its source and extent, DNA damage in mitotic cells results in the execution of the cell death pathway during mitosis, indirect activation of the SAC or thorough activation of the DDR in the following cell cycle. While DNA damage can occur prior to, or independent of, mitotic failure in the context described above, growing evidence indicates that a failure of mitotic progression also induces *de novo* DNA damage during and after mitosis.

DNA damage before the metaphase-anaphase transition

The mechanisms of prometaphase and metaphase failure

The SAC robustly monitors the interaction of kinetochores and spindle microtubules, so that even a single unattached kinetochore is sensed and the SAC activated, leading to prolonged mitotic arrest (*i. e.,* a delay of metaphase-anaphase transition) (46) (Figure 1a). The same effect is achieved by microtubule destabilizing drugs, which include colcemid, nocodazole and vinblastine. The attachment between kinetochore and microtubule is stabilized only when each kinetochore on the sister chromatids is captured by spindle microtubules from the opposite poles so that tension is generated between sister kinetochores, otherwise improper attachment of the kinetochore and microtubule is disrupted by the chromosome passenger complex (CPC), which is composed of INCENP, Borealin, Survivin and Aurora B kinase, and localizes primarily to inner centromeres in prometaphase (47). Thus, inhibition of proper capture of sister kinetochores by polar spindle microtubules also induces SAC activation (48). This type of aberration includes stabilization of microtubule by drugs (*e.g.*, taxol) (49), disruption of cohesin related genes (*e.g.*, cohesin, sororin and shugoshin) (50, 51), centrosome malfunction (*e.g.*, segregation and maturation failure) (49, 52, 53), partial disruption of kinetochore structure proteins (*e.g.*, HEC1 and hNuf2R) (54) and malfunction of genes involved in the stabilization of kinetochore-microtubule attachment (*e.g.*, Polo-like kinase 1) (55) (Figure 1a). Under these conditions cells are considered to be in a prometaphase-like state, since chromosomes are already condensed and not all chromosomes are aligned on the metaphase plate. When the APC or the destruction of APC substrate is suppressed, cells are arrested in metaphase in a SAC-dependent manner (56). Disruption of the function of $p31^{comet}$, a negative regulator of the SAC, has also shown to lengthen the metaphase duration (57). Overexpression of the SAC component, MAD2, causes mitotic arrest, presumably by prolonging metaphase (58). Thus, changing the balance of the activity between the SAC and the APC without disturbing the kinetochore-microtubule attachment causes metaphase arrest (Figure 1b). Inhibition of topoisomerase II-dependent DNA decatenation is another type of insult that leads to metaphase arrest at least in some type of cells (44, 59). This is probably because catenated DNA molecules physically block sister chromatid separation (60), although the molecular details are not well understood.

DNA damage associated with prometaphase and metaphase arrest

It has been described that prolonged arrest in prometaphase causes accumulation of γ–H2AX during and after the arrest (20, 22, 27, 28, 61, 62), which is accompanied by the activation of ATM (63). Although it is controversial (44), topoisomerase II inhibition by ICRF-193 causes the formation of γ–H2AX foci during metaphase arrest (44). Resent findings have demonstrated that these *de novo* DNA damage foci induced by prolonged prometaphase or metaphase arrest are primarily found at chromosome ends (26) (Figure 1). The ends of linear chromosomes, telomeres, are normally protected by a nucleo-protein structure to avoid unwanted activation of DDR pathways (64). Given that telomeric DNA damage has shown to be intrinsically irreparable (65), mitotic telomere deprotection could therefore to be an ideal source of DNA damage signaling and checkpoint activation to ensure cell death or permanent cell cycle arrest, without introducing intrachromosomal DNA breaks. During prolonged mitotic arrest, TRF2, a telomeric protein responsible for telomere protection (66), partially dissociates from telomeres, providing the molecular basis that induces ATM-dependent γ–H2AX foci formation at telomeres (26). The number of telomeric γ–H2AX foci gradually increases during prometaphase arrest, which explains some controversial reports indicating the absence of γ–H2AX foci after transient mitotic arrest (67). Removal of mitotic drugs allows cells to exit mitosis with partially deprotected telomeres, leading to activation of the p53 pathway in the following G1 phase (26–28). Forced progression of the cell cycle after mitotic arrest in IMR-90 normal human fibroblasts by disrupting p53 does not induce telomeric fusion phenotype, suggesting that mitotically deprotected telomeres are resistant to repair (26). However, another group has reported that HCT116 colon cancer cells exposed to microtubule drugs for up to 48 hrs, as well as p53-attenuated IMR-90 cells exposed to nocodazole for 72 hrs, accumulate chromosomal aberrations including dicentric chromosomes (28, 62). Given that cancer cells can delay timely satisfaction of the SAC by several different mechanisms (29), already carry spontaneously dysfunctional telomeres (68) and suffer from aberrant DDR pathways, mitotic telomere deprotection and subsequent cell cycle progression may well be involved in malignant transformation. Recent findings suggest that the DNA damage signal from dysfunctional telomeres in checkpoint compromised cells causes tetraploidization through endoreduplication and/or mitotic failure (69), implying a link between mitotic arrest, telomere deprotection, DNA damage signaling and subsequent tetraploidizaiton.

Inhibition of MPS1 kinase, which is central to the SAC pathway (3), does not suppress telomeric γ– H2AX foci formation (26), which suggests that the SAC pathway is dispensable for the induction of mitotic telomere deprotection, although the involvement of other SAC components need to be experimentally excluded. The telomeric γ–H2AX foci formation is sensitive to the Aurora B inhibitor hesperadin, suggesting that the deprotection of telomeric ends during mitotic arrest is an Aurora B-dependent programmed pathway (26) (Figure 1). How Aurora B kinase induces telomere deprotection during mitotic arrest is still unclear. Active Aurora B at the inner centromere reaches its substrates by diffusion (70), suggesting that telomeric components are less efficient substrates for Aurora B. A recent finding suggests that Aurora B has a potential to directly regulate ATM activity during mitosis (71), although hesperadin-treated mitotic cells exhibit γ–H2AX foci upon irradiation as efficiently as control cells (26). Nevertheless, it is worth noting that inhibition of Aurora B by ZM447439 renders two different colon cancer cell lines resistant to taxol-induced cell death during and after prolonged mitotic arrest (18), which is consistent with the hypothesis that Aurora B-dependent telomere deprotection predisposes cells to initiate a cell death pathway.

DNA damage after the metaphase-anaphase transition

The mechanisms of anaphase, telophase and cytokinesis failure

Once the APC is activated, the purpose of the later phases of mitosis is to distribute sister chromatids to daughter cells correctly, so that single nuclei with identical genetic information are formed in each cell. A failure of this process is evident when segregating chromosomes lag at the anaphase spindle midzone. Such lagging chromosomes potentially cause aneuploidy upon missegregation. Furthermore, even if lagging chromosomes are eventually segregated correctly, they can form a micronucleus that is apart from the main nucleus (7). A leading cause of lagging chromosomes is unresolved merotelic attachment of the kinetochore and microtubules, which occur when a microtubule emanated from both spindle poles becomes attached to a single kinetochore. Because merotelic attachments can satisfy the SAC, anaphase can take place despite their presence (72) (Figure 2). Merotelic attachments are often detected in cells that carry an abnormal number of centrosomes through clustering of multipolar centrosomes into two groups, which act as pseudo-bipolar spindles (23, 24). Recovery from transient prometaphase arrest induced by drugs, or reduction of their rate of correction by the knockdown of the kinesin protein also increases the incidence of merotelic attachments (73). While recovery from transient drug treatment and mitotic progression with extra centrosomes is associated with some degree of mitotic arrest (74), possibly predisposing cells to telomeric DNA damage as described above, premature satisfaction or inhibition of the SAC also causes lagging chromosomes and micronucleus formation without detectable mitotic arrest (Figure 2). Indeed, mouse knockout experiments have shown that a defective SAC pathway induces premature anaphase, lagging chromosomes and micronucleus formation (75–78). Complete knockdown of a kinetochore structural protein that is required for SAC localization induces similar phenotypes (54).

DNA damage associated with lagging chromosome

Recent findings have demonstrated that lagging chromosomes induced by the Mps1 inhibitor Mps1-IN-1 cause *de novo* DNA damage foci formation after the onset of anaphase (79). Lagging chromosomes that are trapped in the cleavage furrow during cytokinesis were often found to be positive for γ–H2AX and MDC1 staining (79) (Figure 2). This phenotype was also found in normally dividing tumor cell lines, such as U2OS, MCF7 and SW480, but the underlying mechanism is not well understood. Chromosomes trapped in the cleavage furrow are proposed to be stabilized by an Aurora B-mediated abscission checkpoint that delays abscission in the presence of unresolved chromosomes (80). Even in the absence of the abscission checkpoint, the physical cutting through chromatin by the abscission machinery, which occurs in yeast (81), does not take place, since the mammalian cleavage furrow regresses rather than cutting through chromatin, causing tetraploid G1 phase cells (80, 82). Given that Aurora B activity is high at the central spindle and midbody (83), where lagging chromosomes localize, an attractive hypothesis that has not been addressed is that DNA breaks on lagging chromosomes also involve Aurora B-dependent telomere deprotection. The consequence of DNA damage on the lagging chromosomes is ATM and CHK2 activation in the following G1 phase, resulting in p53-dependent cell cycle arrest (79). In the absence of p53, these cells keep dividing and exhibit not only an abnormal number of whole chromosomes, but also chromosome translocations, one of the structural chromosomal aberrations associated with tumorigenesis, underscoring the significance of DNA damage induced cell cycle arrest after the generation of lagging chromosomes (79).

DNA damage in the next round of the cell cycle following mitosis and cytokinesis failure

DNA damage associated with mitotic slippage

Cells that have failed during prometaphase and metaphase progression eventually exit mitosis and enter the following G1 phase of the cell cycle, unless they succumb to cell death during mitosis. Mitotic exit without metaphase-anaphase transition, also known as mitotic slippage, occurs through gradual destruction of cyclin B in the presence of an active SAC (10). Usually this results in the formation of a single tetraploid nucleus, or multiple nuclei and micronuclei carrying a 4N genomic content in the following G1 phase (27). G1 cells that have experienced prolonged mitotic arrest potentially contain telomeric DNA damage foci, which they have acquired during mitotic arrest. Gradual accumulation of γ–H2AX after mitotic slippage has also been reported in some human cancer and normal cell lines (20, 61). Such accumulation of γ–H2AX in the following cell cycle is dependent on the activity of caspase (61), and thus should be distinguished from the telomeric γ–H2AX foci formation during mitotic arrest, which is probably caspase-independent (84) (Hayashi and Karlseder, unpublished result). Although there is no direct evidence yet, it is straightforward to hypothesize that telomeric DNA damage caused by mitotic arrest induces caspase activation after the cells exit mitosis and progress into G1 phase, which consequently causes DNA fragmentation with massive γ–H2AX accumulation (85). This hypothesis also applies to the cell death pathway during mitosis. Strong accumulation of γ–H2AX during mitotic arrest is probably a secondary DNA damage that requires prior stimulation, such as telomere deprotection.

DNA damage associated with aneuploidy and micronuclei formation

Cells that fail to segregate their chromosomes correctly after the onset of anaphase potentially exit mitosis without detectable γ–H2AX foci formation, unless lagging chromosomes are trapped in the cytokinesis furrow. If cytokinesis progresses normally, those daughter cells could exhibit a single nucleus carrying an aneuploid genome, or multiple micronulei carrying an abnormal number of chromosomes, as a consequence of the eventual segregation of lagging chromosomes (73) (Figure 3). Aneuploidy has been linked to a transcriptional signature that is related to stress and slow growth (86). Human samples of Down syndrome show an oxidative stress response (87), suggesting the increasing generation of reactive oxygen species (ROS) in aneuploid cells. Recent findings have shown that aneuploid mouse cells induced by SAC deficiency accumulate ROS, which leads to the activation of the ATM/p53 pathway (88). Although a γ–H2AX signal was not detected in those cells, the accumulation of ROS was accompanied by increased oxidative DNA damage (Figure 3), which was measured by the presence of 8-hydroxy-2′-deoxyguanosine (8-OHdG) (88). Although existence of viable human trisomies indicates that aneuploidy does not necessarily cause immediate cell cycle arrest, the degree of aneuploidy may determine the severity of cell fate (86). While gradual accumulation of oxidative DNA damage might induce ATM/p53 pathway after several rounds of cell cycle progression in cells with mild aneuploidy, severe aneuploidy might immediately induce cell cycle arrest or death pathways. Further studies are required to understand the involvement of oxidative and other type of DNA damage in the activation of p53 pathway following aneuploidization.

Micronuclei are known to be frequently positive for γ–H2AX staining (89). A recent study has shown that micronuclei give rise to *de novo* DNA damage focus formation upon entry into next round of the cell cycle (67). Transient exposure of human epithelial cells to nocodazole followed by a mitotic shake-off generated daughter cells carrying micronuclei without a significant increase of γ–H2AX foci. The knockdown of p53 allows those cells to enter the next round of the cell cycle, resulting in the DNA replication-dependent

accumulation of γ– H2AX specifically in micronuclei (Figure 3). The underlying mechanism has been proposed to include defective recruitment of the MCM complex, a DNA replication helicase, and the DNA repair machinery due to inefficient nucleocytoplasmic transport in micronuclei (67). A downside of the DNA damage in micronuclei is chromosome pulverization and a subsequent incorporation of the pulverized chromosome into the main nucleus mass during the following mitosis, which potentially leads to development of cancer (67). It is worth noting, however, that this phenomenon is normally suppressed by the p53-dependent mitotic catastrophe pathway following the first round of mitotic failure.

DNA damage associated with cytokinesis failure

Cytokinesis failure gives rise to two diploid nuclei or multiple micronuclei carrying a 4N genomic content in total within a single cell. As discussed above, DNA trapped in the cytokinesis furrow potentially perturbs abscission. Besides lagging chromosomes, chromatin bridges between sister chromatids in anaphase, owing to the presence of chromosome fusion or unresolved DNA catenanes (90), become an obstacle to the cleavage furrow ingression (Figure 4a). In p53 competent human epithelial cells, most cells with anaphase chromatin bridges complete division upon eventual resolution of the bridges, while a subpopulation of cells becomes binucleated (82).

Cytokinesis failure is not necessarily associated with prolonged mitotic arrest and not necessarily causes DNA damage in the following G1 phase. Indeed, binucleate cells harboring a wild type genetic background induced by a minimum dose of cytochalasin D, an inhibitor of actin polymerization, incorporate BrdU and enter the next mitosis, suggesting the absence of a cell cycle arrest after drug-induced cytokinesis failure (21) (Figure 4a). Other groups came to the same conclusion (5, 22, 28), which strongly argues against the presence of the tetraploidy checkpoint (25). Cytokinesis failure also gives rise to an extra copy of the centrosome, which, in theory, ends up with twice the centrosomes in the next mitosis following centrosome duplication during S phase (91) (Figure 4b). Such supernumerical centrosomes during mitosis are frequently found in many cancer cell lines. Live cell imaging has revealed that they initially form multipolar mitotic spindles, which compromise proper kinetochore-microtubule attachment and delay the metaphase-anaphase transition (23, 24) (Figure 4b). However, extensive mitotic arrest is rarely observed, at least in the cancer cell lines bearing supernumerical centrosomes. Instead, extra centrosomes eventually cluster into two groups, forming pseudo-bipolar spindles with an increased incidence of merotelic attachment (23, 24). As a consequence, those mitotic cells that have failed cytokinesis in the previous cell cycle frequently generate lagging chromosomes and micronuclei, and are thus predisposed to suffer from DNA damage associated with those failures (Figure 4b).

The knockdown of CPC components is also known to cause cytokinesis failure. The CPC changes its localization from the centromere to the central spindle during the metaphase-anaphase transition in order to regulate cytokinesis progression and abscission timing (92). Given that the CPC regulates multiple essential pathway during mitosis, such as chromosome congression and destabilization of incorrectly attached spindle microtubules during prometaphase and cleavage furrow ingression and the abscission checkpoint from anaphase to cytokinesis, inhibition of the CPC causes premature anaphase onset with unaligned chromosomes, which become lagging chromosomes (93–95). Due to the inability to promote cleavage furrow ingression (96) and execute the abscission checkpoint in the presence of lagging chromosomes (80), those cells fail to complete cytokinesis and become tetraploid cells possessing micronuclei, which potentially give rise to DNA damage upon entry into the next round of the cell cycle as described above.

Conclusions

Failures during mitosis and cytokinesis are almost inevitably linked with the generation of cells bearing abnormal ploidy, and are therefore directly linked to transformation. The finding that multiple different pathways lead to *de novo* DNA damage formation during and after those failures shed light on how these cells are removed from the population, although the direct link between DNA damage associated with a specific mitotic failure and cell cycle arrest or cell death needs to be experimentally addressed individually. Considering that an abnormality in DNA ploidy is strongly connected to chromosomal instability, it would not be surprising if there are several independent pathways that lead to p53 and/or caspase activation after different types of mitotic failure. Other pathways that do not involve DNA damage are also suggested to induce p53 activation after prolonged mitotic arrest (97, 98) and cytokinesis failure (99), probably contributing to ensure robust execution of mechanisms that limit the cellular proliferation following those failures.

The primary function of DNA damage associated with mitotic failure is tumor suppression in the presence of intact DNA damage checkpoint pathways. However, it is worth noting that tumor cells often suffer from effects of mitotic failure, including multipolar mitosis, mitotic arrest, lagging chromosomes and micronuclei. In such a checkpoint-compromised background, DNA damage acquired during and after mitotic failures is likely to be propagated, potentially leading to severe DNA rearrangements and cancer progression. In addition, many chemotherapeutic drugs target mitosis to kill cancer cells, and DNA damage likely contributes to execute cell death pathway. Thus, further characterization of molecular pathways that induce DNA damage during and after mitotic failure is vital not only to our understanding of tumorigenesis, but also to the improvement of therapeutic efficacy of the approaches that target mitosis.

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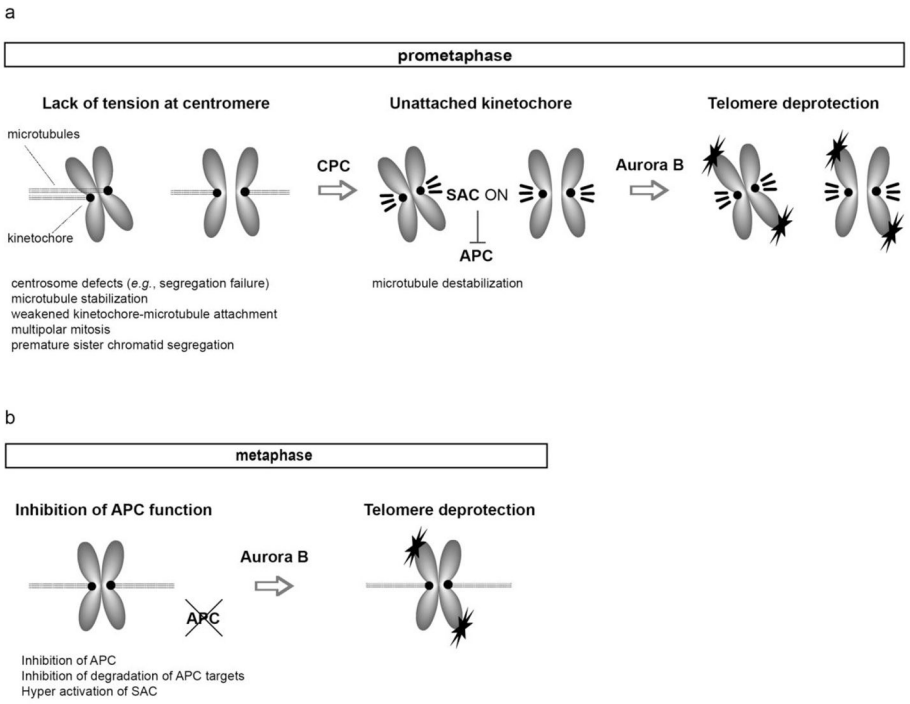

Figure 1.

DNA damage associated with prometaphase and metaphase arrest. (a) Improper attachment of kinetochores and microtubules, which does not generate tension at sister centromeres, is disrupted by the CPC. Possible causes include centrosome segregation failure, microtubule stabilization by drugs, weakened kinetochore-microtubule attachment, multipolar mitosis and premature segregation of sister chromatids (left). This leads to the generation of unattached kinetochores, which serve as a substrate to amplify the SAC signal (middle). The activated SAC suppresses the APC and arrests cells in prometaphase. Unattached kinetochores are also generated by microtubule destabilization (middle). Prolonged arrest in prometaphase induces Aurora B dependent telomere deprotection, leading to DNA damage foci formation at chromosome ends (right). (b) The aberrations that lead to inhibition of the APC function in the presence of proper knetochore-microtubule attachment cause metaphase arrest, which also induces Aurora B dependent telomere deprotection.

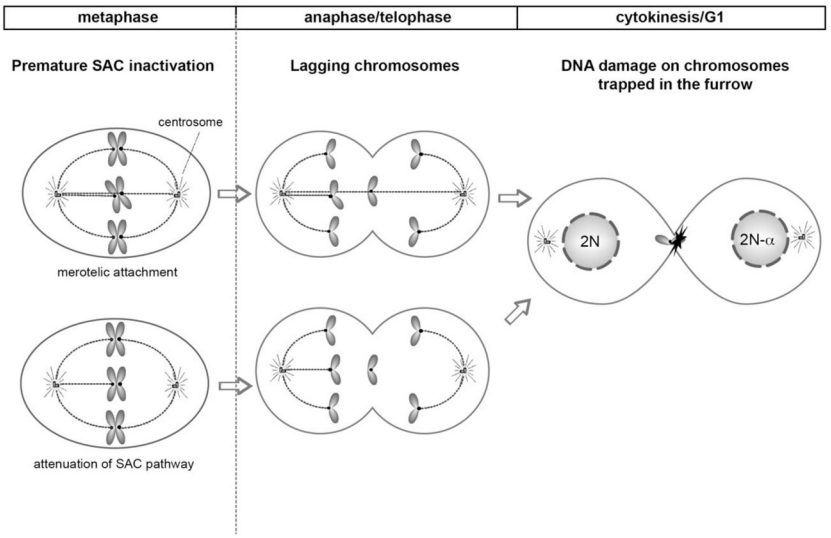

Figure 2.

DNA damage associated with lagging chromosomes. Premature satisfaction or inactivation of the SAC results in the initiation of anaphase in the presence of improperly attached kinetochores (metaphase), which causes lagging chromosome in anaphase and telophase (anaphase/telophase). Those lagging chromosomes that are trapped in the cleavage furrow during cytokinesis accumulate DNA damage foci by unknown mechanisms (cytokinesis).

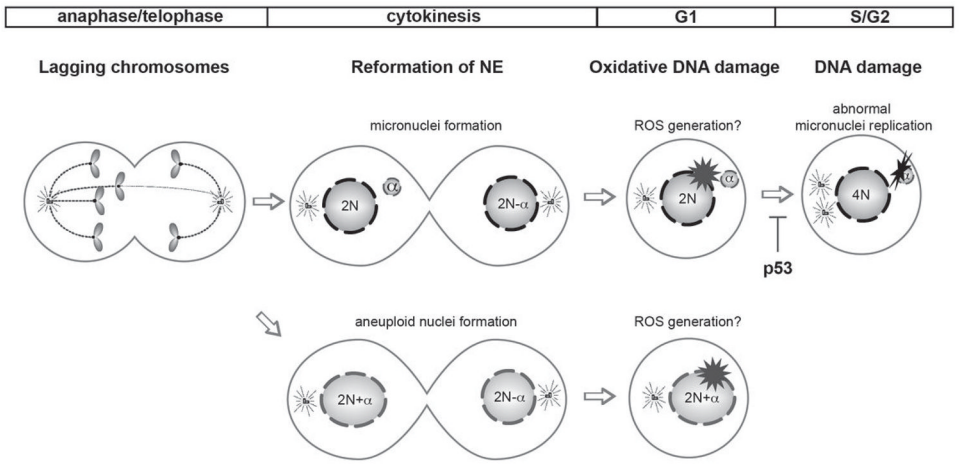

Figure 3.

DNA damage in the next round of cell cycle following mitotic failure and cytokinesis completion. Lagging chromosomes that are not trapped in the cytokinesis furrow can eventually be segregated into daughter cells (anaphase/telophase). Lagging chromosomes can be captured by the main chromosome mass, otherwise nuclear envelope reformation around them generates micronuclei (cytokinesis). Aneuploid nuclei suffer from oxidative DNA damage, presumably because of reactive oxygen species (ROS) generation, which can lead to p53-dependent cell cycle arrest (G1). Forced progression of the cell cycle by p53 attenuation causes abnormal DNA replication of micronuclei, resulting in the formation of *de novo* DNA damage (S/G2).

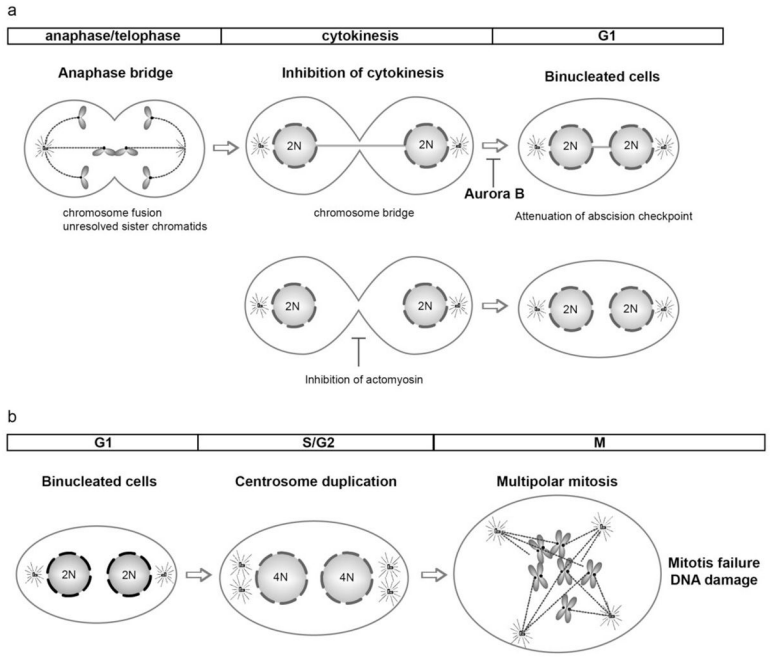

Figure 4.

DNA damage in the next round of the cell cycle following mitosis and cytokinesis failure. (a) Chromosome fusions and unresolved sister chromatids generate chromosome bridges between daughter nuclei (anaphase/telophase). The chromosome bridges inhibit cytokinesis by the Aurora B dependent abscission checkpoint (cytokinesis). Attenuation of the abscission checkpoint or inhibition of furrow ingression by actomyosin drugs causes furrow regression and formation of binucleated cells (G1). (b) Two centrosomes in binucleated cells duplicate during S phase, resulting in binucleated cells possessing four centrosomes in G2 phase (G1, S/G2). Such supernumerical centrosomes can cause multipolar mitosis, which is often associated with cancer cells and frequently generates lagging chromosomes and micronuclei (M). Thus, cells that failed cytokinesis are predisposed to generate DNA damage associated with mitotic failure in the subsequent cell cycle.