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AUTHOR'S VIEW

Different cell fates after mitotic slippage: From aneuploidy to polyploidy

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ABSTRACT

The molecular mechanism responsible for cell fate after mitotic slippage remains unclear. We investigated the different postmitotic effects of aneuploidy *versus* polyploidy using chemical inhibitors of centromere-associated protein-E (CENP-E) and kinesin family member 11 (KIF11, also known as Eg5). Aneuploidy caused substantial proteotoxic stress and DNA damage accompanied by p53-mediated postmitotic apoptosis, whereas polyploidy did not induce these antiproliferative effects.

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Accurate control of chromosome segregation during mitosis is crucial for genomic stability. Chromosome segregation during mitosis involves dynamic interactions between spindle microtubules and kinetochores. To maintain fidelity during chromosome segregation, the spindle assembly checkpoint (SAC) mechanism regulates the proper attachment of microtubules to kinetochores and the tension between the kinetochores of sister chromatids.[1] Antimitotic therapies that target microtubule dynamics, such as taxanes or vinca alkaloids, are widely used in the clinical treatment of cancer.[2] In attempts to improve the therapeutic properties of microtubule inhibitors[3] the non-structural components of microtubules, which are key components of mitosis, have recently attracted attention. Accumulating evidence suggests that the SAC machinery is responsible for the sensitivity of cancer cells to antimitotic agents.[4] Sustained mitotic arrest provides additional opportunities for antimitotic drugs to induce apoptosis.[5] In lesions refractory to antimitotic inhibitors, mitotic slippage induced by SAC down-regulation can bypass prolonged mitotic arrest before activation of the apoptotic pathway and rescue cancer cells from mitotic death.[4,6,7] Given that the SAC mechanism appears to be attenuated in a broad spectrum of primary tumors,[8] it is important to understand the molecular mechanism responsible for cell fate after mitotic slippage. This could help in the development of next-generation mitotic inhibitors and overcome the difficulties associated with treating tumors that are resistant to current antimitotic drugs.

In our recent study published in *Nature Communications*,[9] we investigated the differences in cell fate between aneuploidy and polyploidy after mitotic slippage using 2 distinct mitotic kinesin inhibitors: the centromere-associated protein-E (CENP-E) inhibitor Cmpd-A and the kinesin family member 11 (KIF11, also known as Eg5) inhibitor ispinesib. CENP-E and Eg5 are both mitotic spindle motor proteins of the kinesin superfamily[10] but have distinct molecular mitotic regulatory functions; CENP-E controls the alignment of chromosomes during metaphase, whereas Eg5 regulates the separation of centrosomes and formation of bipolar mitotic spindles. Because of their distinct mitotic functions, inhibition of CENP-E or Eg5 results in distinct postmitotic phenotypes (aneuploidy and polyploidy, respectively) after mitotic slippage under an SAC-defective condition.[9] This experimental model using siRNAs or chemical inhibitors of CENP-E and Eg5 was used to evaluate differences in the postmitotic effects between aneuploidy and polyploidy. We demonstrated that aneuploidy, but not polyploidy, is responsible for postmitotic apoptosis mediated by tumor protein p53 (TP53, best known as p53).[9] We also demonstrated that aneuploidy generates both replication stress-mediated double-stranded breaks (DSBs) and proteotoxic stress, which activate the DNA damage response (DDR) and unfolded protein response (UPR) pathways (Fig. 1).[9] Thus, we propose that the integrated DDR and UPR signaling pathways play important roles in eliminating chromosome instability. However, 2 key questions remain to be addressed. First, it is unclear how aneuploidy-mediated DDR and UPR interact

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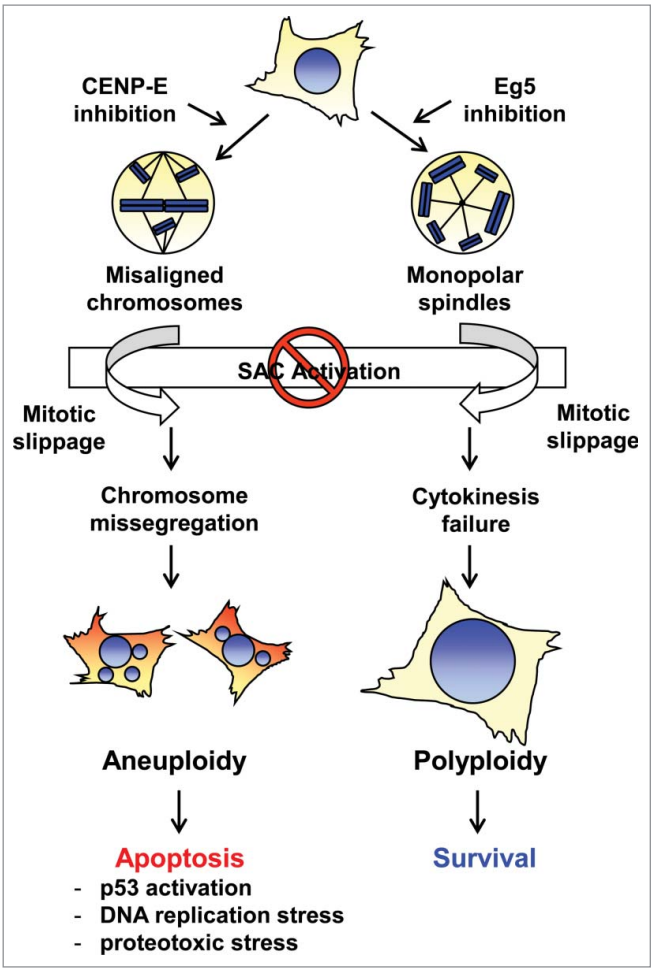

Figure 1. Different cell fates after mitotic slippage: from aneuploidy to polyploidy. Inhibition of centromere-associated protein-E (CENP-E) or kinesin family member 11 (KIF11, also known as Eg5) results in misaligned chromosomes and monopolar spindle formation respectively during mitosis, resulting in mitotic death through activation of the spindle assembly checkpoint (SAC). However, under SAC-impaired conditions, chromosome misalignment leads to aneuploidy through chromosome missegregation after mitotic slippage, whereas monopolar spindle formation leads to polyploidy through cytokinesis failure. Aneuploidy, but not polyploidy, induces postmitotic apoptosis in a tumor protein p53 (TP53, best known as p53)-dependent manner, concurrent with DNA replication stress and proteotoxic stress.

functionally. Assuming that rapid changes in chromosome copy number markedly alter cellular protein homeostasis, aneuploidy induced substantial proteotoxic stress in our model, including pathologic changes in the endoplasmic reticulum, aggresome formation, and transcriptional activation of UPR genes (spliced form of X-box binding protein 1 [XBP1] and autophagic vesicle-associated form of microtubule-associated protein 1 light chain 3B [MAP1LC3B-II, also known as LC3B-II]). DNA replication stress was induced collaterally, with downregulation of the replication regulatory proteins mini-chromosome maintenance complex component 2 (MCM2) and cyclin A.[9] Thus, activation of the UPR in response to aneuploidy-associated proteotoxic stress might downregulate the protein translation machinery for these replication components, leading to the generation of DSBs. Second, it is unclear how aneuploidy-mediated proteotoxic stress is functionally associated with activation of the p53 pathway. Aneuploidy concurrently induced proteotoxic stress and p53 activation in our model.[9] Although these findings suggest that proteotoxic stress controls the p53 pathway, presumably via the UPR signaling pathway, the mechanisms underlying this functional interaction remain unclear. Further studies are needed to clarify the mechanistic interactions between the p53 and UPR signaling pathways during aneuploidy-mediated postmitotic apoptosis.

Our findings also indicate that aneuploidy-mediated postmitotic effects could be used to develop therapeutic strategies for cancer, such as induction of unequal chromosome distribution in cancer cells to induce potent antiproliferative and cell death effects. Thus, the CENP-E inhibitor, which exhibits an aneuploidy-mediated postmitotic antiproliferative effect in SAC-impaired cells, could have potential as a next-generation cancer therapeutic drug.[9] In our study, immunohistochemical analysis using a tumor microarray revealed that the SAC hub protein BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B, also known as BUBR1) was downregulated in approximately 60% of primary tumors tested (44/70).[9] Although the SAC activities of these tumors are unclear, it is possible that some of these primary tumors downregulate the SAC machinery to escape mitotic death under treatment with a mitotic inhibitor. However, given its potent postmitotic antiproliferative activity after mitotic slippage, the CENP-E inhibitor could be potentially effective in these refractory tumors and would broaden the range of existing cancer therapeutics. In this case, p53 played an important role in the postmitotic antiproliferative effect of the CENP-E inhibitor in SAC-impaired cancers. In such cancers, accumulation of p53 in patients with *Tp53* wild-type tumors might be a potential predictive biomarker for treatment with this drug and patients with *Tp53*-mutant tumors could be excluded. In conclusion, our recent study demonstrated differences in postmitotic antiproliferative effects between aneuploidy and polyploidy after mitotic slippage. These findings shed light on the importance of developing next-generation antimitotic inhibitors and might advance clinical strategies through the identification of appropriate biomarkers for these drugs.

Disclosure of potential conflict of interest

Akihiro Ohashi is an employee of Takeda Pharmaceutical Company Ltd.

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