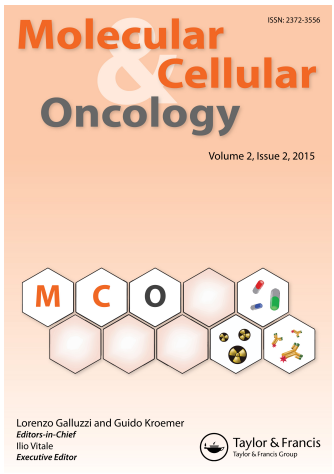

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Sustaining the Spindle Assembly Checkpoint to improve cancer therapy

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Sustaining the Spindle Assembly Checkpoint to improve cancer therapy.

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Abstract

To prevent chromosome segregation errors, the Spindle Assembly Checkpoint (SAC) delays mitosis exit until proper spindle assembly. We found that the FCP1 phosphatase and its downstream target WEE1 kinase oppose SAC, promoting mitosis exit despite malforme spindles. We further showed that targeting this pathway could be useful for cancer therapy.

Keywords

FCP1; WEE1 inhibitor; Spindle Assembly Checkpoint; SAC; Taxane; Vinca alkaloid; apoptosis.

Abbreviations

SAC	Spindle Assembly Checkpoint
CDK1	Cyclin-dependent kinase 1
AMCDs	Anti-Microtubule Cancer Drugs
MCL-1	Myeloid cell leukemia 1

Mitosis is the fastest phase of the cell division cycle. Nevertheless, the safeguard mechanism Spindle Assembly Checkpoint (SAC) can delay mitosis exit until proper spindle formation.[1] Through still unclear mechanisms, however, an exceeding mitosis prolongation can translate into activation of the apoptotic programme.[2] During the normal timing of mitosis, the cyclin B-dependent kinase (CDK) 1 operates antiapoptotic measures like inhibitory phosphorylation of caspases.[3] However, as time of mitosis is extended (for instance in case spindle assembly is hampered somehow), a progressive degradation of the antiapoptotic myeloid cell leukemia (MCL) 1 protein appears to change drammatically cell sensitivity to apoptotic cell death.[3,4] Indeed, it has been shown that impairing mitosis exit by depleting cells of CDC20, the crucial ubiquitin-ligase coactivator required for mitotic cyclins degradation and CDK1 inactivation, thus even in absence of spindle defects, promotes a prolonged mitotic arrest that ends up in a deadly fate.[5]

Exposure of cells, expecially aneuploid cancer cells, to drugs that impair microtubule physiology and spindle assembly, like the widely used anti-microtubule cancer drugs (AMCDs) taxanes and vinka alcaloids, induces a SAC-dependent mitotic delay. At therapeutic concentrations, AMCDs appear to induce a very transient mitotic delay in cancer cells. Some cells die in mitosis but other appear to exit from mitosis despite malformed spindles.[4,6] This is due to an adaptation-like mechanism by which cancer cells slip through SAC and exit mitosis abnormally and prematurely in the absence of a correctly assembled spindle. Slipped, and even more aneuploid, cells either stop dividing or die at later stages, however, it is possible they that may give rise to resistant clones.[4,6] A recently developed model suggests that, during AMCDs-induced prolonged mitosis, proapoptotic signals accumulate but cells may survive if they slip through mitosis before a certain proapoptotic signal threshold has been reached. Conversely, cells die if the threshold is reached before slippage.[4,6] Thus, a better understanding of how cells slip through AMCDs-activated SAC may provide clues to improve AMCDs efficacy in cancer cell killing.

By studying the mechanisms of mitosis exit, we previosly reported a novel, transcription-independent, and crucial role for the essential RNA polymerase II-carboxy-terminal domain phosphatase FCP1 in bring about CDK1 inactivation at the end of mitosis.[7] We identified cyclin degradation pathway components, like CDC20 and USP44, a deubiquitinating enzyme, and the CDK1 inhibitory kinase WEE1 as crucial FCP1 targets.[7] At mitosis exit, FCP1 dephosphorylated WEE1, reactivating it to dampen down CDK1 activation, and CDC20 and USP44, to promote ubiquitin-dependent cyclin B degradation.

Recently, we analysed the FCP1 relevance in SAC slippage and sensitivity to therapeutic AMCDs concentrations.[8] We found that FCP1 affected SAC slippage, mitosis exit and cell death in the presence of AMCDs. Depleting FCP1 protracted the time cells spent in mitosis in the presence of

AMCDs.[8] In addition, in FCP1-depleted AMCDs-treated cells, the levels of MCL-1 protein substantially decreased during prolonged mitosis and signficantly higher rates of apoptotic cell death were induced.[8] In addition, we found that WEE1 was reactivated in an FCP1-dependent manner during prolonged mitosis in AMCDs-treated cells and had a crucial role in promoting SAC slippage by lowering CDK1 activity, otherwise required to sustain the SAC.[9] Indeed, genetic or chemical WEE1 downregulation significantly extended mitosis and promoted cell death in several AMCDs-treated cancer cell lines and primary human adult lymphoblastic leukemia cells. Thus, the FWC (FCP1-WEE1-CDK1) pathway opposes the SAC and promotes slippage under AMCDs regimens. On the contrary, its inhibition prolongs mitotic duration, proapoptotic signals accumulation and eventually cell death in AMCDs-treated cancer cells.[8]

WEE1 kinase is known to control the onset of mitosis by performing inhibitory phosphorylation of CDK1. WEE1 is also a crucial kinase that prevents mitosis onset in case cells have incompletely replicated or damaged DNA. Since it has been observed that forcing cells with damaged DNA into mitosis strongly promotes cell death, WEE1 inhibitors have been produced with the intent of an anticancer combination therapy with DNA damaging drugs and an orally available one is currently in clinical trials.[10] Besed on our findings, we hypothesize that inhibiting WEE1 under AMCDs treatment would promote more efficient cancer cell killing by delaying slippage, thus, increasing the chances for proapoptotic signals accumulation (see Figure 1). Given the avaiability of a clinically usable WEE1 inhibitor, we sugget that it would be worth to perform clinical trials in which the WEE1 inhibitor is combined with AMCDs-based cancer therapy. Such a therapeutic combination would be particularly important in those clinical settings in which AMCDs are used as monotherapeutic agents as in the case of prostate cancer and several solid and hematological malignancies as second line treatments.

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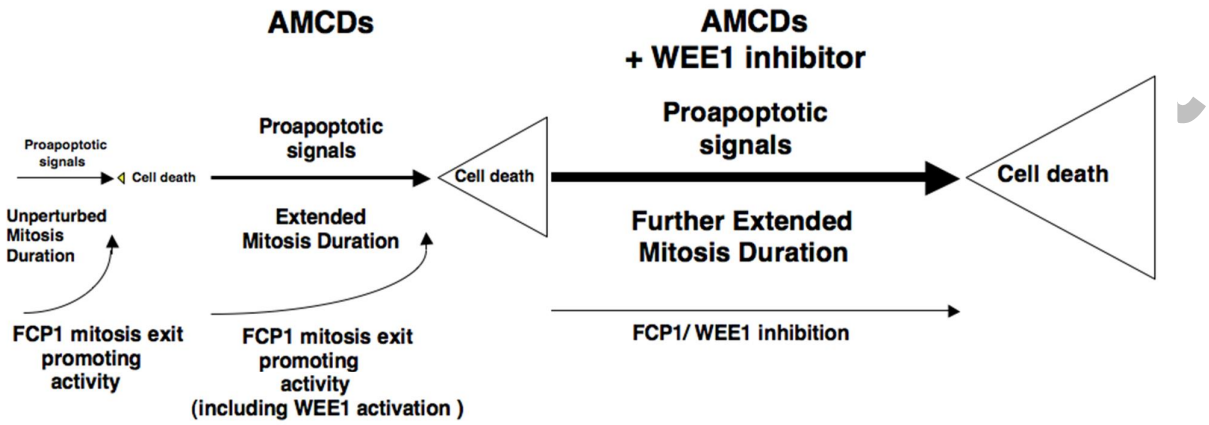

Figure 1 Impact of FCP1/WEE1 inhibition on anti-microtubule cancer drugs (AMCDs) treatment. The FCP1 phosphatase promotes mitosis exit during unperturbed mitosis as well as slippage through an AMCDs-activated spindle assembly checkpoint (SAC). Inhibiting FCP1 or its downstream WEE1 kinase can delay slippage, further extend mitosis and, giving proapoptotic signals more time to accumulate (or more time for degradation of antiapoptotic signals), increase chances for a deadly fate of AMCDs-treated cancer cells.