Molecular & Cellular Oncology 2:2, e969658; April/May/June 2015; Published with license by Taylor & Francis Group, LLC

# The complexity of life and death decisions in mitosis

Laura A Diaz-Martinez* and Hongtao Yu*

Howard Hughes Medical Institute; Department of Pharmacology; University of Texas Southwestern Medical Center; Dallas, TX USA

**Keywords:** mitosis, apoptosis, taxol, cell death, mitochondrial fusion

The anticancer drug taxol stabilizes microtubules and activates the spindle checkpoint, causing prolonged mitotic arrest in cancer cells. Our recent work suggests that the cellular decision to live or die following mitotic arrest is a complex process involving crosstalk between competing apoptotic and adaptation pathways.

The natural product taxol is the most commonly prescribed anticancer drug.[1] Taxol binds to and stabilizes microtubules, resulting in mitotic spindle defects and mitotic arrest in human cells.[1] This mitotic arrest depends on the spindle checkpoint, which prevents premature chromosome segregation and mitotic exit through inhibition of the anaphase-promoting complex/cyclosome (APC/C).[2] Once the spindle defects are corrected the checkpoint is extinguished, leading to APC/C activation, degradation of cyclin B1 (CCNB1), and mitotic exit. The spindle defects caused by taxol result in chronic activation of the checkpoint and prolonged mitotic arrest. Cells then either die through the intrinsic apoptosis pathway or exit mitosis abnormally through mitotic slippage or adaptation.[3]

Using elegant live-cell imaging to observe cell fate decisions, the Taylor and Mitchison labs discovered tremendous heterogeneity in the cellular response to taxol among different cancer cell lines, and even among different subpopulations of the same line.[4,5] Furthermore, in cell lines that primarily undergo apoptosis following mitotic arrest, inhibition of apoptosis nudges cells toward adaptation. Conversely, in cell lines that primarily undergo adaptation, depletion of the APC/C activator cell division cycle 20 (CDC20) blocks adaptation and promotes apoptosis.[5] Thus, the specific fate of a given cell after prolonged mitotic arrest is the result of competition between apoptosis and adaptation pathways.

Recent work from our laboratory extends these findings and uncovers feedback mechanisms between the apoptosis and adaptation pathways.[6] Our findings paint a more complicated picture of cell fate decisions during mitotic arrest. The concurrent execution of 2 intertwined, competing pathways underlies the heterogeneous cellular response to taxol.

## Two Ways to Exit Mitotic Arrest: Apoptosis and Adaptation

Through a genome-wide small interfering RNA (siRNA) screen in HeLa cells, we systematically identified factors in human cells that mediate the cellular response to taxol.[6] As expected, these factors belong to the spindle checkpoint network and the intrinsic mitochondrial apoptotic network, among other networks. We were intrigued by the lack of involvement of other molecular pathways (e.g., autophagy) in the taxol response, and tested whether apoptosis and adaptation were the only 2 major exit pathways for mitotically arrested cells. Mitotic duration in the presence of taxol varies depending on the cell type, but does not typically last longer than 20 hours. After this prolonged mitotic arrest, cells either undergo adaptation or apoptosis, at least in the cell lines we tested. Blocking both adaptation and apoptosis caused cells to arrest in mitosis for over 60 hours.[6] This striking result suggests that apoptosis and adaptation are the only 2 major fates for a cell arrested in mitosis. Indeed, autophagy appeared to be suppressed during taxol-triggered mitotic arrest in RPE1 cells (**Fig. 1A**).

It has been argued that the apoptosis and adaptation pathways act independently of each other during mitotic arrest.[7] Depending on which pathway is executed faster, cells preferably undergo apoptosis or adaptation. For example, adaptation is expected to be the faster process and the more frequent fate in U2OS and RPE1 cells. A strong prediction of this "molecular race" model is that blocking the slower apoptosis pathway should not have a major effect on the kinetics of the faster adaptation pathway. In contrast to this expected outcome, depletion of 2 key apoptosis regulators BCL2-associated X protein (BAX) and BCL2-antagonist/killer 1 (BAK1) in U2OS or RPE1 cells lengthens mitotic duration and delays


*Correspondence to: Laura A Diaz-Martinez; Email: ladiazmartinez@utep.edu; Hongtao Yu; Email: hongtao.yu@utsouthwestern.edu
Submitted: 08/28/2014; Revised: 09/01/2014; Accepted: 09/02/2014
http://dx.doi.org/10.4161/23723548.2014.969658

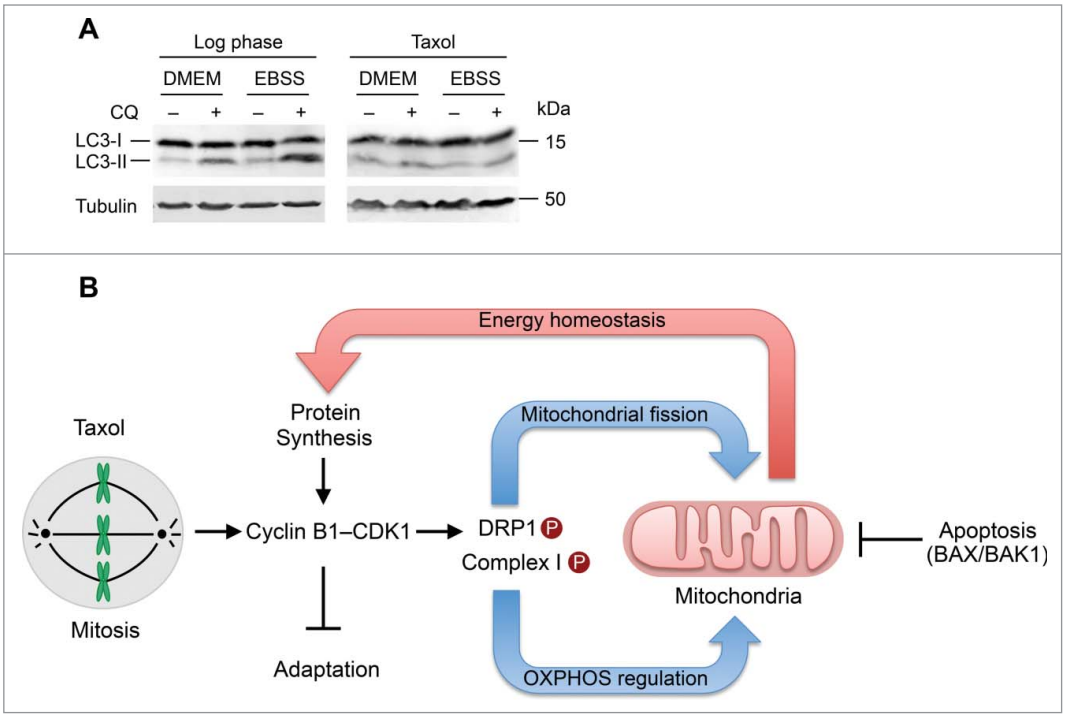


**Figure 1.** Mitochondria-dependent crosstalk between apoptosis and adaptation in response to mitotic arrest. (**A**) Autophagy is suppressed during taxol-induced mitotic arrest. Lysates from log-phase or taxol-arrested RPE1 cells in normal medium (Dulbecco's Modified Eagle Medium; DMEM) or under starvation conditions (Earl's Balanced Salt Solution; EBSS) in the presence or absence of chloroquine (CQ) were blotted with anti-LC3 (MAP1LC3A/B) or anti-tubulin (as loading control) antibodies. The positions of LC3-I and LC3-II (as an indicator of autophagy) are indicated. Unlike log-phase cells, taxol-treated cells did not show an increase in LC3-II formation in response to starvation and CQ, suggesting that autophagy might be suppressed under these conditions. (**B**) Cartoon depicting crosstalk between apoptosis and adaptation pathways through the mitochondria. The master mitotic kinase cyclin B1 (CCNB1)–cyclin dependent kinase 1 (CDK1) regulates mitochondrial fission and activity by phosphorylating dynamin 1-like protein (DNM1L or DRP1) and components of Complex I. Conversely, mitochondrial activity might impact mitotic events by providing energy for spindle dynamics, chromosome movement, signaling cascades, and the continuous transcription and translation of mitotic proteins, such as cyclin B1. During a prolonged mitotic arrest, whether a cell undergoes apoptosis or adaptation is likely a result of complicated interplay between these pathways. Diminishing cyclin B1 levels during adaptation will likely produce changes in mitochondrial structure and function that affect apoptosis. Mitochondrial permeabilization triggered during apoptosis may impact cyclin B1 translation and facilitate adaptation.

adaptation.[6] This unexpected result suggests the existence of crosstalk between the apoptosis and adaptation pathways during mitotic arrest. Disrupting the apoptosis pathway at different steps further reveals that this crosstalk occurs upstream of mitochondrial outer membrane permeabilization (MOMP), an initiating event during apoptosis.[6]

## How do Mitochondrial and Mitotic Events Influence One Another?

Although our work implicates the involvement of mitochondria in coupling mitotic apoptosis and adaptation, the detailed mechanisms remain elusive. Mitochondrial morphology and function undergo dynamic changes during the cell cycle. In mitosis, phosphorylation of dynamin 1-like (DNM1L, best known as DRP1) by cyclin B1–cyclin-dependent kinase 1 (CDK1) promotes mitochondrial fission.[8] Cyclin B1-–CDK1 also modulates mitochondrial function during G2/M by phosphorylating components of the respiratory chain.[9] Intriguingly, we showed that, similar to BAX/BAK1 depletion, depletion or chemical inhibition of DRP1, a dynamin-like mitochondrial fission factor, also delayed adaptation in U2OS cells.[6] Our results suggest that the converse might also be true: i.e., mitochondrial fission and function might regulate mitosis. Modulation of mitochondrial fission or fitness can influence the duration of taxol-induced mitotic arrest.

One mechanism by which mitochondrial function could affect adaptation is by influencing cyclin B1 production. Continuous cyclin B1 transcription and translation are required to sustain a mitotic arrest.[10] Impairment of mitochondrial fitness during a prolonged mitotic arrest, as a result of continual DRP1-mediated fission or partial mitochondrial permeabilization by proapoptotic proteins, could compromise energy homeostasis and impact protein translation. Thus, mitochondrial fitness might be a coupling factor between the apoptosis and adaptation pathways (**Fig. 1B**).

Alternatively, components of the mitochondrial pathway could directly influence mitotic processes via physical interactions or post-translational modifications. For example, cyclin B1–CDK1 has been detected in the mitochondrial matrix,[9] and might thus be regulated directly by mitochondrial proteins. We also cannot rule out other possibilities, including mitochondria-independent roles for BAX, BAK1, or DRP1 in mitosis.

In conclusion, our recent findings reveal an unexpected crosstalk between mitotic apoptosis and adaptation. Future studies aimed at determining the nature of this coupling will help us understand the cell-killing mechanisms of the antimitotic chemotherapeutic drugs, and ultimately lead to new strategies that can uncouple the cell death and survival pathways and increase the efficacy of these widely used antitumor drugs.

## References


1. Wani MC, Horwitz SB. Nature as a remarkable chemist: a personal story of the discovery and development of taxol. Anticancer Drugs 2014; 25: 482-7; PMID:24413390; http://dx.doi.org/10.1097/CAD.0000000000000063
2. Jia L, Kim S, Yu H. Tracking spindle checkpoint signals from kinetochores to APC/C. Trends Biochem Sci 2013; 38: 302-11; PMID:23598156; http://dx.doi.org/10.1016/j.tibs.2013.03.004
3. Gascoigne KE, Taylor SS. How do anti-mitotic drugs kill cancer cells? J Cell Sci 2009; 122: 2579-85; PMID:19625502; http://dx.doi.org/10.1242/jcs.039719
4. Gascoigne KE, Taylor SS. Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. Cancer Cell 2008; 14: 111-22; PMID:18656424; http://dx.doi.org/10.1016/j.ccr.2008.07.002
5. Huang HC, Shi J, Orth JD, Mitchison TJ. Evidence that mitotic exit is a better cancer therapeutic target than spindle assembly. Cancer Cell 2009; 16: 347-58; PMID:19800579; http://dx.doi.org/10.1016/j.ccr.2009.08.020
6. Diaz-Martinez LA, Karamysheva ZN, Warrington R, Li B, Wei S, Xie XJ, Roth MG, Yu, H. Genome-wide siRNA screen reveals coupling between mitotic apoptosis and adaptation. EMBO J 2014; 33:1960-76; PMID:25024437; http://dx.doi.org/b10.15252/embj.201487826
7. Huang HC, Mitchison TJ, Shi J. Stochastic competition between mechanistically independent slippage and death pathways determines cell fate during mitotic arrest. PLoS One 2010; 5: e15724; PMID:21203573; http://dx.doi.org/10.1371/journal.pone.0015724
8. Taguchi N, Ishihara N, Jofuku A, Oka T, Mihara K. Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. J Biol Chem 2007; 282: 11521-9; PMID:17301055; http://dx.doi.org/10.1074/jbc.M607279200
9. Wang Z, Fan M, Candas D, Zhang TQ, Qin L, Eldridge A, Wachsmann-Hogiu S, Ahmed KM, Chromy BA, Nantajit D, et al. Cyclin B1/Cdk1 coordinates mitochondrial respiration for cell-cycle G2/M progression. Dev Cell 2014; 29: 217-32; PMID:24746669; http://dx.doi.org/10.1016/j.devcel.2014.03.012
10. Mena AL, Lam EW, Chatterjee S. Sustained spindle-assembly checkpoint response requires de novo transcription and translation of cyclin B1. PLoS One 2010; 5: e13037; PMID:20927403; http://dx.doi.org/10.1371/journal.pone.0013037