RESEARCH HIGHLIGHT

How CHKing ROS signaling preserves genomic integrity

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In a recent study published in *Cell*, Zhang et al. broadly identify proteins modified during oxidative stress induced by H_2O_2 or chemotherapy drugs and establish the DNA damage protein CHK1 as a key regulator of mitochondrial ROS.

Oxidants are double-edged swords. At low concentrations, reactive oxygen species (ROS), including the archetype H_2O_2, facilitate key biological processes, including proliferation and defense against infectious agents in an "oxidative eustress" environment. In contrast, high ROS levels drive aberrant signaling and cause damage to DNA, protein, and lipids, resulting in a state referred to as "oxidative distress."[1] How this oxidative distress affects various cellular components to alter biological functions is incompletely understood.

Previous studies have demonstrated that several anticancer drugs induce oxidative distress, which contributes in varying degrees to their antineoplastic effects. Earlier characterizations of the proteomic changes that occur in cells in oxidative distress have been relatively incomplete, focusing on a small number of proteins or failing to connect modifications to functional consequences. In the May issue of *Cell*, Junbing Zhang and colleagues start to fill in these gaps by integrating findings from a global analysis of cysteine modifications with a genome-wide CRISPRi screen to systematically identify ROS-regulated targets that impact anticancer drug action.[2]

Using the isobaric tandem-mass tag approach, the authors generated a comprehensive portrait of cysteine modifications induced by 11 anticancer drugs previously reported to induce ROS. Of the detected cysteines, 2498 localized to four distinct clusters of reactive cysteines, with the thioredoxin reductase inhibitor auranofin (AUR) and NAD(P)H:quinone reductase inhibitor β-lapachone causing the largest number of cysteine modifications. Further inspection of the modified cysteines revealed a strong link to multiple ribosomal proteins in line with the general effects of cytotoxic drugs on processes such as cell growth, proliferation, and apoptosis. Comparison to changes induced by the oxidant H_2O_2 and prevented by the antioxidant N-acetylcysteine (NAC) demonstrated that proteomic changes after AUR treatment have the greatest overlap with those induced by H_2O_2 and can potentially be applied to study redox biology more broadly.

To extend these findings from chemical proteomics, the authors performed a CRISPRi screen for modifiers of AUR-induced antiproliferative effects and identified 66 genes contributing to resistance, including genes encoding the DNA damage-activated kinases ATR and CHK1,[3,4] and 51 genes contributing to sensitization, including multiple genes encoding mitochondrial ribosomal proteins. Integrating this CRISPRi screen with the global proteomics, the authors identified CHK1 as a nuclear sensor of AUR-induced oxidative damage (Fig. 1). In particular, AUR caused CHK1 activation, as manifested by its autophosphorylation at Ser296[5] in an NAC-inhibitable manner. However, instead of promoting CHK1 activation through ATR-mediated phosphorylation of the CHK1 kinase domain as stalled replication forks do,[3,4] AUR caused oxidation of Cys408 in the C-terminal autoinhibitory domain of CHK1[6] to sulfenic acid, a modification that is sufficient to activate CHK1 under cell-free conditions. Importantly, CHK1 inhibition or depletion resulted in elevated nuclear H_2O_2 levels during oxidative stress, indicating the importance of CHK1 activation in the cellular response to ROS.

Although CHK1 is best known for its ability to phosphorylate CDC25A, Tresslin, and KAP2 after replication forks stall,[7] thereby facilitating fork stabilization and replication restart,[3,4] Zhang et al. showed that CHK1 plays a different role in resolving oxidative distress. Comparison of CHK1 phosphoproteomic data and AUR CRISPRi results identified Ser67 of SSBP1, a protein involved in replication of mtDNA, as a previously unrecognized CHK1 substrate. Further analysis showed that CHK1-mediated phosphorylation results in exclusion of SSBP1 from mitochondria, leading to decreased mtDNA content, decreased expression of mtDNA-encoded components of the electron transport chain (ETC), decreased mitochondrial ROS production, decreased nuclear ROS levels, and partial rescue from AUR-induced antiproliferative effects. Importantly, all of these effects of oxidative CHK1 activation were mimicked by SSBP1 downregulation, replacement of wild-type SSBP1 with an S67D phosphomimetic mutant that does not enter mitochondria, or pharmacological inhibition of mitochondrial protein synthesis by doxycycline. Conversely, CHK1 inhibition resulted in increased mitochondrial SSBP1, increased mtDNA-encoded ETC components, and increased nuclear ROS.

The importance of this newly identified nucleus-to-mitochondrion signaling pathway is further supported by the observation that SSBP1 downregulation protects cells from additional anticancer drugs that elevate ROS, including arsenic trioxide, β-lapachone and cisplatin. Moreover, SSBP1 is decreased in cell lines selected for cisplatin resistance and in clinical ovarian cancer that has relapsed after platinum-containing chemotherapy, suggesting that the CHK1-SSBP1 pathway might contribute to clinical drug resistance.

As the complex regulatory networks that respond to elevated ROS come into focus, what can we surmise about the relationships between components of DNA damage response pathways and ROS? First, the unexpected identification of CHK1 as an H_2O_2

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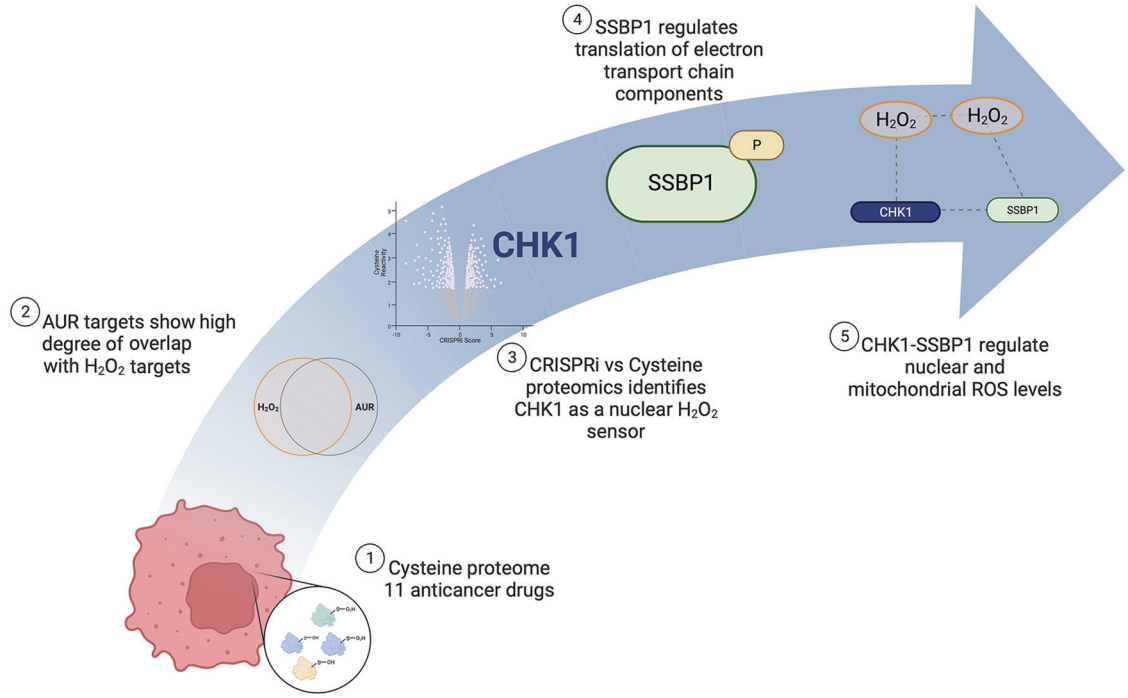

Fig. 1 Discovery of CHK1 as a master nuclear and mitochondrial H_2O_2 regulator. Cysteine proteomics of 11 anticancer drugs identified AUR as a close mimetics of H_2O_2. The combination of cysteine proteomics, phosphoproteomics and a functional CRISPRi screen identified a CHK1-SSBP1 module as a regulator of nuclear and mitochondrial ROS levels.

sensitive enzyme that modulates further H_2O_2 generation indicates that cellular ROS have distinct regulators that modulate oxidative stress at the subcellular level. This is a major step forward in understanding of redox signaling, which has previously been painted with a much broader brush. Second, it is likely that further surprises await us. The CRISPRi screen of Zhang et al. also suggested that ATR and BRCA2 contribute to AUR resistance, but their distinct roles remain to be elucidated. Because ATR does not seem to be required for H_2O_2-induced CHK1 activation, the mechanisms by which ATR and BRCA2 help resolve oxidative stress are likely to be novel and interesting. Third, while Zhang et al. identified CHK1 as a key sensor that responds to a specific oxidant, H_2O_2, and modulates the antiproliferative effects of multiple antineoplastic agents, regulation of ROS might be even more complex than we now realize. For example, multiple sensors might be involved in multiple redox networks and there might be relays where signals are passed from one oxidant target to another, eventually leading to modifications of key targets.[8] Fourth, while the authors combined "omic" approaches to identify a unique H_2O_2 response pathway, one cannot help but wonder whether future investigations should also integrate the extracellular biology of ROS signaling and its potential contribution to anticancer drug action. Despite technical challenges in oxidant profiling, the groundbreaking study of Zhang et al. indicates that it is possible to define clear determinants of anticancer drug-mediated ROS signaling. We look forward to future studies that describe the cell biology of other reactive species (e.g., reactive nitrogen species) in equally exquisite detail and continue to elucidate the systems biology of oxidative distress.

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ADDITIONAL INFORMATION

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