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RESEARCH ARTICLE

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ATR prevents Ca^{2+} overload-induced necrotic cell death through phosphorylation-mediated inactivation of PARP1 without DNA damage signaling

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Abstract

Hyperactivation of PARP1 is known to be a major cause of necrotic cell death by depleting NAD^+/ATP pools during Ca^{2+} overload which is associated with many ischemic diseases. However, little is known about how PARP1 hyperactivity is regulated during calcium overload. In this study we show that ATR kinase, well known for its role in DNA damage responses, suppresses ionomycin, glutamate, or quinolinic acid-induced necrotic death of cells including SH-SY5Y neuronal cells. We found that the inhibition of necrosis requires the kinase activity of ATR. Specifically, ATR binds to and phosphorylates PARP1 at Ser179 after the ionophore treatments. This site-specific phosphorylation inactivates PARP1, inhibiting ionophore-induced necrosis. Strikingly, all of this occurs in the absence of detectable DNA damage and signaling up to 8 hours after ionophore treatment. Furthermore, little AIF was released from mitochondria/cytoplasm for nuclear import, supporting the necrotic type of cell death in the early period of the treatments. Our results reveal a novel

Abbreviations: AIF, apoptosis inducing factor; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; ATR, ataxia telangiectasia and Rad3 related; BSA, bovine serum albumin; DAPI, 4′, 6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; DDR, DNA damage responses; DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; FBS, fetal bovine serum; Glut, glutamate; HMGB1, high mobility group box 1; IP, immunoprecipitation; IPed, immunoprecipitated; KD, kinase dead; MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; NAC, N-acetyl-L-cysteine; NAD^+, nicotinamide adenine dinucleotide; PBS, phosphate-buffered saline; PAR, poly (ADP-ribose); PARP1, Poly [ADP-ribose] polymerase 1; PARylation, poly ADP-ribosylation; PI, propidium iodide; PLA, proximity ligation assay; QA, quinolinic acid; ROS, reactive oxygen species; U2OS, human osteosarcoma cell line; VE-822, ATR kinase inhibitor; WB, western blot; WT, wild type; Z-VAD-FMK, apoptotic cell death inhibiton.

Zhengke Li and Hui Wang-Heaton are co-first authors.

related to the phosphorylation of PARP1 by ATR as presented in Figure 7. Z. Li wrote the manuscript draft. H. Wang-Heaton, Z. Li and P.R. Musich also participated in experimental design and manuscript preparation. B.M. Cartwright, Y. Makinwa, B.A. Hilton, N. Shkriabai, M. Kvaratskhelia, and S. Guan were involved in generating some of the experimental data and supporting manuscript preparation. Q. Chen and X. Yu generated and provided genetically modified cells critical to this study. Y. Zou is the senior author who oversaw and directed this study. P.R. Musich also provided help in directing this study.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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