A disease-relevant mutation of SPOP highlights functional significance of ATM-mediated DNA damage response

Received: 17 June 2020 Revised: 29 September 2020 Accepted: 12 October 2020
Published online: 15 January 2021
S119N knock-in PC-3 cell lines. As shown in Fig. 1j, SPOP Serine 119 mutants (S119A or S119N) suppressed SPOP-induced MCM3 degradation and ubiquitination. In addition, as shown in Supplementary Fig. S4c, the IR-induced dissociation SPOP from MCM3 was abrogated in the absence of ATM. These results strongly support the conclusion that ATM-mediated SPOP Serine 119 phosphorylation is required for dissociation of SPOP-MCM3 and degradation of MCM3.

To strengthen the conclusion on the functional significance of ATM phosphorylation of Serine 119, we conducted
Fig. 1 ATM-mediated SPOP Serine 119 phosphorylation is required for the DNA damage response in prostate cancer. a Cellular radiosensitivity was measured with the colony formation assay in LN-Cap cells. b Cell cycle analysis of PC-3 cells expressing the mutant SPOP. c Micronuclei quantification of PC-3 cells expressing the mutant SPOP, assessed by cell sorting. d Expression of γH2AX, HA, and α-Tubulin in PC-3 cells transiently transfected with HA-SPOP constructs, assessed by Western blotting. e In cell interaction was interrogated via the Proximity Ligation Assay. f In vitro interaction of ATM and SPOP was assessed by biolayer interferometry. g ATM Kinase activity was quantified by the in vitro kinase assay followed by measuring the production of ADP. h Pull-down of HA-SPOP by Co-IP in wild type and S119N cell lines after treated with 5Gy IR. i Pull-down of HA and MCM5 and MCM3 by co-immunoprecipitation. PC-3 cells transfected with vector, wild type, or the mutant SPOP constructs were treated with 5Gy of IR. j Immunoblotting of ubiquitination of MCM3 in SPOP WT, S119A and S119N knock-in cell lines. k Tumor volume of xenograft tumors post radiation. l Relative growth delay of tumors after radiation. m Schematic illustration of the model for the ATM-SPOP-MCM3 signaling in prostate cancer cells.

Radiosensitivity experiments for prostate cancer xenografts in athymic nude mice. We found that xenografts expressing the S119A mutant were significantly more sensitive as compared to wild-type or vector (Fig. 1k). The specific tumor growth delay rate for S119A is 2.7 while wild-type was 1.2 (p = 0.0001) (Fig. 1l). These results strongly support that the ATM phosphorylation of SPOP on Serine 119 is critical for reducing radiosensitivity.

In conclusion, we demonstrate that a prostate cancer-relevant mutation of SPOP on Serine 119 causes prolonged DNA repair and hypersensitivity to ionizing radiation. We prove that Serine 119 is required for the SPOP interaction with ATM, and demonstrate that ATM phosphorylates SPOP on Serine 119. Further, we identify the MCM5-MCM3 complex is among SPOP interacting proteins in response to DNA damage. We demonstrate that S119 phosphorylation is required for the SPOP-MCM3 dissociation and it inhibits MCM3 ubiquitination and degradation (Fig. 1m). Taken together, we highlight a novel DDR pathway mediated by ATM phosphorylation of SPOP. These findings have clinical impact for prostate cancer patients with SPOP mutations, as DNA damaging therapies may be particularly effective in this subgroup. This also provides the first evidence for a pathophysiological relevant mutation linked to ATM phosphorylation in the DDR.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China [81672743, 81974464 and 81972861], Beijing-Tianjin-Hebei Basic Research Cooperation Project [19JZDJC64500(Z)], the Alabama Innovation Fund, from the Ministry of Science and Technology [2016YFC0904601], Shenzhen Basic Research Project (JCYJ20160331114230843), and Tianjin Medical University Cancer Institute and Hospital Innovation Fund [1803].

AUTHOR CONTRIBUTIONS

B.X. designed the study; M.X., J.S.F., J.M., Q.Z., and R.J.B. performed the experiments; Y.S., F.M., and Y.M. performed the statistical analysis; M.X., J.S.F., R.X., and B.X. wrote the manuscript. All authors read and approved the final manuscript.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41392-020-00381-7) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

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