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Control of replication stress and mitosis in cancer stem cells

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Introduction

Cancer stem cells (CSCs) are a cell subpopulation within the tumor mass which drives colorectal cancer (CRC) development, relapse and resistance to therapy. Depleting CSC ensures the eradication of CRC, which, in advanced stages, has limited therapeutic options. Here, we delved into the replication stress response (RSR) in colorectal CSCs with the aim of identifying novel therapeutic vulnerabilities for the design of effective CRC-CS-targeting strategies.

Material and Methods
We used multiple (cyto)genetically-characterized patient-derived CRC-SCs intrinsically resistant to ATR/CHK1 inhibitors (ATRi/CHK1i) or acquiring resistance by prolonged CHK1i administration. RSR proficiency/robustness were evaluated by drug-screening assays and cytofluorimetry, while molecular mechanisms of DNA replication and RSR by genomic and proteomic analyses followed by immunostaining-based validation, and by DNA fiber and other replication assays. Prevention of CHK1i resistance, ATRi/CHK1i sensitization and additional RSRi were assessed by apoptotic and drug resistance assays and/or analyzing CSC-derived tumor growth in xenografted NSG mice.

Results and Discussions
We showed that the RSR is efficient in CRC-SCs as we described unique roles for PARP1 and MRE11/RAD51. First, we demonstrated that PARP1 is upregulated in CRC-SCs resistant to several replication poisons, including conventional CRC chemoterapeutics, and to RSR inhibitors (RSRi). In these cells, PARP1 modulates replication fork speed resulting in low constitutive RS levels. Second, we provided evidence that MRE11 and RAD51 cooperate in the genoprotection and mitosis execution of PARP1-upregulated CRC-SCs. Importantly, such roles represent a therapeutic vulnerability for CRC-SCs. Indeed, PARP1i sensitized CRC-SCs to ATRi/CHK1i, killing them via replication catastrophe, and prevented the development of resistance to CHK1i. Moreover, MRE11i + RAD51i selectively killed PARP1-upregulated CRC-SCs via mitotic catastrophe dependent on caspases.

Conclusion
CHK1i resistance in CRC-SCs is mediated by PARP1 upregulation, and can be reverted and/or prevented by PARP1 inhibition or concomitant RAD51-MRE11 targeting. These results provide the rationale for biomarker-driven clinical trials in CRC using distinct RSRi combinations.