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Genomic Instability

Major finding: Depletion of nuclear RPA by unscheduled origin firing triggers replication fork breakage.

Concept: The amount of single-stranded DNA generated upon ATR loss exceeds the amount of available RPA.

Impact: Surplus RPA protects replication forks and prevents irreparable DNA damage during replication stress.

EXHAUSTION OF RPA LEADS TO REPLICATION CATASTROPHE

When DNA replication forks stall, the dissociation of the replication fork helicase from DNA polymerase generates replication protein A (RPA)-coated single-stranded DNA (ssDNA), which leads to the activation of Ataxia Telangiectasia and Rad3-Related (ATR) kinase. ATR signaling prevents genomic instability by preventing collapse and breakage of stalled replication forks and suppressing firing of new replication origins throughout the nucleus, but it is unclear how these functions are related. After inhibiting ATR in cells with stalled replication forks, Toledo and colleagues unexpectedly observed a substantial lag between ATR inhibition and replication fork breakage, suggesting that an ATR-independent mechanism can protect replication forks for a limited time. Quantitative image-based cytometry analysis of immunofluorescently labeled single cells revealed that in the context of replication stress, ATR inhibition leads to DNA double-strand breaks specifically in cells where ssDNA generation exceeds the accumulation of chromatin-bound RPA. Together with the findings that RPA overexpression and suppression of replication origin activity each prevented replication fork breakage, this observation suggested that unscheduled origin firing and subsequent ssDNA generation caused by ATR loss exhausts the nuclear supply of RPA and promotes genome-wide replication fork breakage. The "replication catastrophe" caused by exhaustion of RPA irreversibly arrested cell-cycle progression and induced senescence due to irreparable DNA damage, with forced premature chromosome condensation revealing breaks in replicating loci within every chromosome. In addition to providing an explanation for how the local and global functions of ATR in preventing genomic instability are linked, these findings suggest that RPA exists in excess of the amount required for normal DNA replication to shield ssDNA and protect against replication fork breakage during replication stress. Because oncogenic transformation is associated with replication stress, these findings also predict that cancer cells might have a limited RPA pool and be sensitive to agents that induce replication catastrophe. ■

Toledo LI, Altmeyer M, Rask MB, Lukas C, Larsen DH, Povlsen LK, et al. ATR prohibits replication catastrophe by preventing global exhaustion of RPA. Cell 2013;155:1088–103.

Signaling

Major finding: SIN1 phosphorylation disrupts mTORC2 and impairs AKT activation in response to various stimuli.

Mechanism: Phosphorylation of both Thr86 and Thr398 by S6K1 or AKT induces SIN1 dissociation from mTORC2.

Impact: Cancer-associated mutations in *SIN1* promote hyperactive mTORC2/AKT signaling and tumor growth.

A NEGATIVE FEEDBACK MECHANISM REGULATES mTORC2 ACTIVITY

The mTOR pathway functions to promote protein translation and cell growth through two distinct multiprotein complexes, mTORC1 and mTORC2, which phosphorylate the downstream substrates ribosomal protein S6 kinase 1 (S6K1) and AKT, respectively. mTORC1 can negatively regulate mTORC2 activity in part via phosphorylation of insulin receptor substrate 1 (IRS1) and growth factor receptor-bound protein 10 (GRB10), but additional mechanisms by which mTORC1 modulates mTORC2/AKT signaling are incompletely understood. Liu and colleagues found that mTORC1 activation inhibited mTORC2 signaling by promoting phosphorylation of SIN1 (also known as mitogen-activated protein kinase-associated protein 1), a unique and essential component of mTORC2. SIN1 phosphorylation at both Thr86 and Thr398 was cell context dependent and mediated by AKT in adipocytes and largely by S6K1 in epithelial cells. S6K1-driven SIN1 phosphorylation triggered the dissociation of SIN1 from mTORC2 following physiologic stimulation with various growth factors, including insulin, insulin-like growth factor 1, EGF, and platelet-derived growth factor, thereby impairing mTORC2 complex assembly; phosphorylation of both residues was required to disrupt the interaction of SIN1 with Rictor and the mTOR kinase domain. In addition, SIN1 phosphorylation specifically suppressed mTORC2-induced AKT activation and decreased cell viability following treatment with etoposide or cisplatin, supporting a role for S6K1-dependent SIN1 regulation in inhibiting cell survival. Intriguingly, an ovarian cancer–associated mutation in SIN1, Arg81Thr (R81T), attenuated SIN1 phosphorylation at Thr86, resulting in prolonged interaction of SIN1 with mTORC2 and sustained activation of AKT signaling in response to various growth factors. Furthermore, expression of SIN1-R81T enhanced anchorage-independent growth and tumor formation, suggesting that this gain-of-function mutation allows SIN1 to bypass S6K1-dependent inhibition of mTORC2-driven cell proliferation. These results identify a negative feedback mechanism by which mTORC1/S6K1 signaling attenuates mTORC2/AKT activity independently of IRS1 and GRB10, and suggest that dysregulation of SIN1 phosphorylation contributes to tumorigenesis. ■

Liu P, Gan W, Inuzuka H, Lazorchak AS, Gao D, Arojo O, et al. Sin1 phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorigenesis. Nat Cell Biol 2013;15:1340–50.

AACR American Association for Cancer Research

CANCER DISCOVERY

Exhaustion of RPA Leads to Replication Catastrophe

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