

RESEARCH WATCH

Ewing Sarcoma

Major finding: *EWSR1-ETS* fusions can arise by a sudden burst of complex, loop-like rearrangements termed chromoplexy.

Clinical relevance: Chromoplexy-generated fusion genes are linked to aggressive disease in Ewing sarcoma.

Impact: The identification of chromoplexy-generated fusions elucidates a mechanism by which sarcoma may develop.

EWING SARCOMA GENE FUSIONS CAN BE GENERATED VIA CHROMOPLEXY

Structural rearrangements that result in gene fusions occur commonly in cancer and can drive tumorigenesis. Sarcomas are often defined by characteristic gene fusions, such as the *EWSR1-ETS* fusions in Ewing sarcoma (where *EWSR1* is fused to one of the ETS transcription factors, *FLI1*, *ERG* or *ETV1*). The downstream effects of *EWS-FLI1* and other sarcoma fusion proteins have been widely studied, but the timing and mechanism by which they occur have not been determined. Anderson and colleagues investigated the genesis of *EWSR1-ETS* fusions through whole-exome or whole-genome sequencing data from 124 patients with Ewing sarcoma. As has been previously reported, the tumors were genetically quiet with few somatic mutations. However, analysis of structural rearrangements revealed that in 52 of 124 (42%) tumors, the *EWSR1-ETS* fusion arose by chromoplexy, a sudden burst of complex, loop-like rearrangements, in contrast to more the common mechanism of fusion gene generation by simple reciprocal translocation. Chromoplexy-generated fusions were enriched in patients with aggressive disease and poor outcomes. Chromoplexy-mediated

generation of *EWSR1-ETS* fusions occurred preferentially in bursts in early replicating DNA and in transcriptionally active regions. The *EWSR1-ETS* fusion was always at the center of the loops but multiple other genes were simultaneously disrupted. Analysis of matched relapsed or metastatic tumors revealed that primary and relapsed tumors diverged after the generation of *EWSR1-ETS* in the ancestral clone, with primary and relapsed tumors evolving independently. Further, recurrent chromoplexy-generated fusions were detected in chondromyxoid fibroma, synovial sarcoma, and phosphaturic mesenchymal tumor, exhibiting a similar looped formation. In addition to elucidating the mechanisms by which Ewing sarcoma and other tumor-associated fusion genes are generated, these findings provide insight into the mutational processes underpinning Ewing sarcoma. ■

Anderson ND, de Borja R, Young MD, Fuligni F, Rosic A, Roberts ND, et al. Rearrangement bursts generate canonical gene fusions in bone and soft tissue tumors. *Science* 2018;361:eaam8419.

Cell Cycle

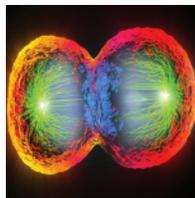
Major finding: Cells possess an ATR-enforced intrinsic checkpoint controlling the S/G₂ transition.

Concept: The S/G₂ transition is marked by a switch-like phosphorylation of FOXM1 to initiate the mitotic program.

Impact: Regulation of the S/G₂ transition by ATR promotes genome integrity.

AN S/G₂ CHECKPOINT REGULATED BY ATR PRESERVES GENOME INTEGRITY

Progression through the cell cycle is tightly regulated by a series of regulatory checkpoints. The G₁/S, G₂/M, and metaphase/anaphase transition checkpoints have been well characterized, but a checkpoint controlling the S/G₂ transition has not been identified. DNA damage can activate the checkpoint kinase ATR to arrest cells in the S or G₂ phase and ensure complete replication before mitosis, prompting Saldivar and colleagues to hypothesize that ATR may regulate the S/G₂ transition. Inhibiting ATR in S phase accelerated mitotic entry, but ATR inhibition did not accelerate mitotic entry in G₂ cells, suggesting that ATR acts in S phase to delay mitosis. However, S-phase shortening was not sufficient to explain the combined shortening of S and G₂, and it was determined that, in addition to the role in S phase, ATR controlled accumulation of prometotic factors to regulate G₂ duration. ATR inhibition resulted in premature accumulation of cyclin B in S phase as well as other prometotic factors, indicating that ATR suppresses transcription of G₂/M genes poised for activation during S phase. Analysis of publicly available chromatin immunoprecipitation sequencing data revealed that the transcription



factor FOXM1, which is frequently overexpressed in cancer, was enriched at the prometotic genes and drove premature expression of cyclin B. Inhibiting ATR in early S phase led to hyperphosphorylation of FOXM1, resulting in premature FOXM1 activation and early mitosis. Mechanistically, FOXM1 was phosphorylated by CDK1 at the S/G₂ transition, and this phosphorylation switch facilitated G₂ entry. In contrast, during normal DNA replication, ATR was activated by ETAA1, and prevented CDK1-dependent FOXM1 phosphorylation until G₂. Thus, ATR inhibition deregulated the S/G₂ transition to promote early mitosis, leading to underreplicated DNA and DNA damage. Collectively, these findings uncover an S/G₂ checkpoint regulated by ATR that is responsible for maintaining genome integrity. As ATR inhibitors are being tested in cancer clinical studies, this finding could shed light on strategies to improve the efficacy of ATR inhibitors in the clinic. ■

Saldivar JC, Hamperl S, Bocek MJ, Chung M, Bass TE, Cisneros-Soberanis F, et al. An intrinsic S/G₂ checkpoint enforced by ATR. *Science* 2018;361:806–10.

CANCER DISCOVERY

Ewing Sarcoma Gene Fusions Can Be Generated via Chromoplexy

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