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Immunotherapy

Major finding: Aberrant splicing can produce retained intron neoepitopes that are presented by MHC 1.

Approach: A computational method predicts intron retention neoepitopes that can be validated by mass spectrometry.

Impact: Discovery of intron retention neoepitopes in melanoma may aid development of personalized cancer vaccines.

RETAINED INTRONS CAN PRODUCE TUMOR-SPECIFIC NEOEPITOPES IN MELANOMA

Tumor-specific neoepitopes may be therapeutic targets in personalized cancer vaccines and immunotherapies. The targets can be neoepitopes generated by somatic mutations or aberrant peptides generated from germline antigens that are transcriptionally silenced in normal adult tissues. Cancer transcriptomes often contain aberrant mRNAs resulting from dysregulated splicing, a process which frequently results in intron retention. Transcripts with retained introns are translated and degraded by the nonsense-mediated decay pathway. This generates peptides that may be presented on the cell surface by MHC class I; however, it is not known if retained intron transcripts yield tumor-specific neoepitopes in cancer. Smart, Margolis, and colleagues developed a computational method to detect intron retention events using bulk RNA-sequencing (RNA-seq) data from tumors and identify putative retained intron neoepitopes. This *in silico* approach was applied to tumor RNA-seq data from two cohorts of patients with melanoma (48 patients total) treated with immune checkpoint inhibitors. The cohorts had comparable levels of intron retention and predicted retained intron neoepitopes. Across cohorts, mean total predicted neoepitope load, comprised of both somatic mutation neoepitopes and retained intron neoepitopes, was augmented roughly 0.7-fold with the addition of retained intron neoepitopes. Retained intron neoepitope load was not associated with response to immune checkpoint blockade. Mass spectrometry immunopeptidome analysis of multiple cancer cell lines determined that several predicted retained intron neoepitopes were processed and presented on MHC 1 on the surface of melanoma cells, validating these retained intron-derived peptides as tumor neoepitopes. Taken together, these findings suggest that intron retention from aberrant splicing can produce immunogenic peptides that are presented by MHC 1. Further, the computational method created to identify tumor-specific retained intron neoepitopes may aid in the development of personalized cancer vaccines. ■

Smart AC, Margolis CA, Pimentel H, He MX, Miao D, Adeegbe D, et al. Intron retention is a source of neoepitopes in cancer. Nat Biotechnol 2018 Aug 16 [Epub ahead of print].

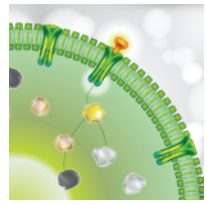
Signaling

Major finding: Oncogenic mutations and targeted drugs promote slow deactivation of activated RAS signaling.

Approach: Combined live-cell microscopy and optogenetic profiling characterize the dynamics of RAS–ERK signaling.

Impact: Mutation-dependent changes in signal transmission dynamics drive signal misinterpretation in cancer cells.

MUTATIONS AND TARGETED DRUGS ALTER RAS SIGNALING DYNAMICS IN CANCER



Multiple signaling transduction pathways converge to form complex networks that drive essential cellular processes. Oncogenic mutations have been shown to drive aberrant activation of crucial signal transduction pathways, but it is unclear whether these mutations affect the kinetics of pathway activation. Recently, optogenetic methods in which light inputs are used to selectively activate intracellular signaling nodes have developed to separately characterize the effect of a specific node on a shared node. To further elucidate the role of oncogenic mutations and targeted drugs on the dynamics of RAS–ERK signaling, Bugaj and colleagues performed live-cell or high-throughput fluorescence microscopy to assess the effect of optogenetic stimulation of RAS on ERK output in normal lung epithelial, patient-derived non–small cell lung cancer (NSCLC), and NIH3T3 cell lines. Compared to NIH 3T3 cells, the NSCLC cell line H1395, which harbored a mutation in the P-loop of *BRAF* that enhances BRAF dimerization, exhibited a delay in both activation and deactivation of ERK in response to optogenetic RAS stimulation. H1395 cells were less responsive than normal lung epithelial cells or NSCLC cells harboring the BRAF kinase domain mutation $BRAF^{V600E}$ to high-frequency RAS stimulation due to the slow ERK pathway kinetics. Consistent with these findings, NSCLC cell lines harboring *BRAF* P-loop mutations exhibited slow ERK activation in response to optogenetic stimulation of RAS, and treatment with paradox-activating BRAF dimerization-promoting drugs prolonged ERK kinetics in normal cells. Slowed pathway kinetics induced by BRAF paradox inhibitors drove altered expression of ERK target genes in response to pulsed RAS stimulation, and these expression changes promoted cell-cycle entry compared to cells with normal, fast ERK pathway kinetics. Together, these results elucidate the role of cancer mutations and targeted drugs in altering cellular signaling dynamics and suggest that characterization of signaling kinetics in cancer cells may provide insight into cancer biology and therapeutic strategies. ■

Bugaj LJ, Sabnis AJ, Mitchell A, Garbarino JE, Toettcher JE, Bivona TG, et al. Cancer mutations and targeted drugs can disrupt dynamic signal encoding by the Ras-Erk pathway. Science 2018;361:eaao3048.

AACR American Association for Cancer Research

CANCER DISCOVERY

Mutations and Targeted Drugs Alter RAS Signaling Dynamics in Cancer

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