Programmable and selective silencing of oncogenic fusion transcripts by CRISPR-Cas13b via breakpoint sequence recognition

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Background: Oncogenic gene fusions are drivers of cancer, yet most remain undruggable by therapeutic approaches. CRISPR-Cas13 systems offer a programmable strategy for RNA targeting, but their application to oncogenic fusion transcripts remains unexplored. Here, we establish CRISPR-PspCas13b as a personalized platform for silencing diverse oncogenic fusion RNAs. Method: We programmed PspCas13b to recognize fusion breakpoint sequences in cancer cells and evaluated breakpoint-directed RNA targeting across distinct cancer models. We applied this strategy to wild-type and drug-resistant fusion variants, including BCR::ABL1 variants with tyrosine kinase inhibitor (TKI) resistance and disease relapse. Cas13b components were delivered using mRNA and lipid nanoparticles (LNPs), and target RNA and protein depletion, RNA processing, transcript integrity, and cellular responses were assessed using quantitative PCR, western blotting, flow cytometry, short- and long-read RNA sequencing, and mass spectrometry-based proteomics. Results: Recognition and cleavage of fusion breakpoint sequences by PspCas13b selectively depleted fusion transcripts and reduced oncoproteins while sparing homologous wild-type transcripts. Analyses revealed that Cas13b-mediated cleavage generated aberrant, translation-incompetent RNA species, including transcripts containing internal deletions proximal to the cleavage site, revealing consequences of targeted RNA cleavage. In the BCR::ABL1 model, breakpoint-directed PspCas13b efficiently silenced both wild-type and drug-resistant BCR::ABL1 variants, including mutations associated with TKI resistance and disease relapse. Molecular profiling demonstrated suppression of BCR::ABL1 tyrosine kinase signaling and transcriptomic and proteomic remodeling following Cas13b-mediated silencing. These changes culminated in erythroid differentiation and apoptosis of drug-resistant leukemia cells, whereas approved TKIs were ineffective against these variants. The same personalized design framework enabled efficient silencing of additional oncogenic fusion transcripts, demonstrating broad applicability across genetically distinct cancers. Conclusion: Collectively, these findings establish PspCas13b as a versatile programmable RNAtargeting platform and provide a molecular framework for precise and personalized targeting of undruggable and drug-resistant oncogenic fusion transcripts.