Engineered CRISPR-Cas12a for combinatorial functional genomics in prostate cancer

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Background:

Combination therapies are highly sought after in oncology and have been highly successful in some well-known examples involving 3 or more combinations, such as lymphoma. However, for a given N-plex combination, exhaustive testing of all possible 3 or more combinations of therapeutic targets is challenging using conventional approaches, due to the exponential explosion of the number of possible combinations (2^N). Here I describe an engineered CRISPR-Cas12a functional genomics tool we recently developed that enables testing numerous 6-plex combinations of genes and non-coding genomic elements for their impact on phenotype in human cells. To compress such combinatorial screens to avoid exhaustive testing, I propose a framework known as group testing, conceptually related to pooled COVID testing. In pilot studies we have shown feasibility of applying these approaches to prostate cancer cell models. Future studies in our lab will focus on applications of these approaches to identify combinations of genetic perturbations to prostate cancer cells that can trigger systemic anti-tumor immunity.

Methods:

Inspired by prior biophysics literature on Cas12a, we engineer an Acidaminococcus Cas12a (AsCas12a) variant, multiplexed transcriptional interference AsCas12a (multiAsCas12a), that incorporates R1226A, a mutation that stabilizes the ribonucleoprotein–DNA complex via DNA nicking.

Results:

The multiAsCas12a-KRAB fusion improves CRISPRi activity over DNase-dead AsCas12a-KRAB fusions, often rescuing the activities of lentivirally delivered CRISPR RNAs (crRNA) that are inactive when used with the latter. multiAsCas12a-KRAB supports CRISPRi using 6-plex guide arrays in high-throughput pooled screens. Using multiAsCas12a-KRAB, we discover enhancer elements and dissect the combinatorial function of cis-regulatory elements in human cells. These results instantiate a group testing framework for efficiently surveying numerous combinations of chromatin perturbations for biological discovery and engineering. We showed that the multiAsCas12a platform supports multiplexed transcriptional targeting in prostate cancer cell models.

Conclusions:
We developed and benchmarked multiAsCas12a CRISPRi as a new combinatorial functional genomics platform that paves the way for future studies of combinatorial genetic dependencies in broad biological contexts, including prostate cancer cell models.

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Conflicts of Interest Disclosure
C.C.H., C.M.W., R.D. and L.A.G. have filed patent applications related to multiAsCas12a. J.S. is a scientific consultant for Treeline Biosciences. L.A.G. has filed patents on CRISPRof/on and CRISPR functional genomics and is a co-founder of Chroma Medicine. The other authors declare no competing interests.