

WHOLE GENOME CRISPR-CAS9 SCREENS IN A CANCER CELL LINE PANEL CO-CULTURED WITH ANTIGEN-SPECIFIC CYTOTOXIC CD8 T CELLS ARE A POWERFUL ENGINE FOR IMMUNO-ONCOLOGY DRUG TARGET DISCOVERY

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Background Cytotoxic T lymphocytes (CTLs) are a key driver of the anti-tumor immune response. Understanding the molecular mechanisms mediating this process can reveal therapeutic targets whose pharmacological inhibition can increase responses to immunotherapy. In recent years, forward genetic screens with CRISPR-Cas9 have been successfully applied to study the interaction between tumor cells and CTLs *in vitro*. Here, we extend this approach to a broad panel of human cancer cell lines and define methods for prioritization and validation of immuno-oncology therapeutic targets.

Methods A panel of seven HLA-A*02 cancer cell lines were prioritized to span multiple cancer lineages. Tumor cells were infected with a whole-genome CRISPR-Cas9 library and co-cultured with primary human CD8⁺ T cells expressing the NYESO1 HLA-A*02-restricted T cell receptor. Next-generation sequencing and statistical analysis were used to define the top genes that affected tumor cell killing by CTLs. We validated the top hits from the screen *in vitro* and developed an adoptive cell transfer model to validate the sensitizing effects *in vivo*.

Results Comparison of screen hits uncovered both known and novel pathways that sensitize tumor cell lines to CTL killing, including surface checkpoint molecules, epigenetic regulators, genes that control cytokine response, autophagy, post-transcriptional regulation, and cell surface glycosylation. We prioritized genes for validation based on effect size, druggability, and TCGA correlation between target expression and an immune-deficient tumor microenvironment. We showcase our approach using the known immune regulator, PTPN2. PTPN2 knockout in tumor cells sensitized them to CTL-mediated killing in co-culture assay *in vitro* and adoptive cell transfer model *in vivo*.

Conclusions Our cancer cell and T cell co-culture CRISPR screening platform revealed multiple known as well as novel genes to comprehensively characterize the mechanisms regulating tumor cell killing by CTLs, providing a rich resource of therapeutic targets to advance into drug discovery.

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