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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ABSTRACT

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 *(1)*. 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 *(2)*. PTPN2 knockout in tumor cells sensitized them to CTL-mediated killing in co-culture assay in vitro and adoptive cell transfer (ACT) 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.

SCREENING STRATEGY AND T CELL ENGINEERING

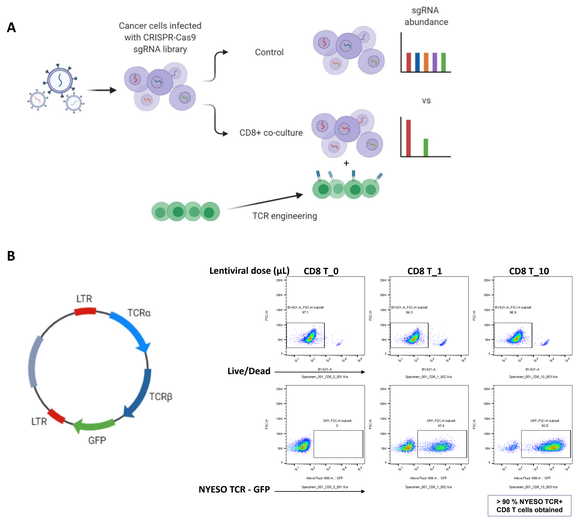
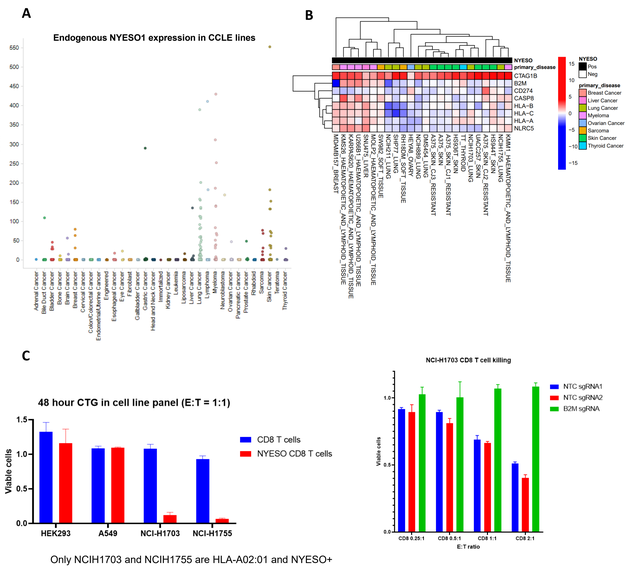


Figure 1: T cell co-culture platform. (A) Schematic diagram of screening strategy (B) Vector schematic diagram and confirmation of transgene expression in NYESO TCR+ CD8 T cells isolated from healthy human donors.

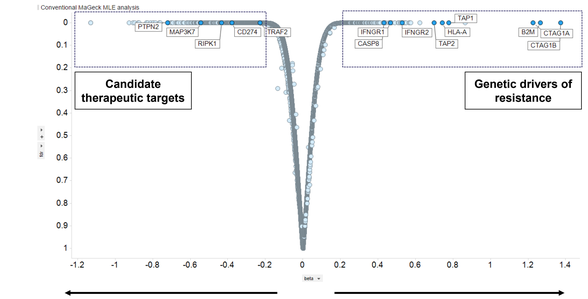
CANCER CELL LINE SELECTION



Only NCIH1703 and NCIH1755 are HLA-A02:01 and NYESO+

Figure 2: Cancer cell line selection for co-culture screens. (A) Lineage restriction of endogenous NYESO1 (CTAG1A/B) expression in CCLE cell lines. (B) MHC-I expression in HLA-A02:01, NYESO positive cell lines. Cell lines with correct haplotype but no endogenous NYESO expression were also identified and engineered with lentiviral-delivered NYESO transgene. (C) In vitro co-culture validation of antigen-specific killing of select cell lines.

RESULTS



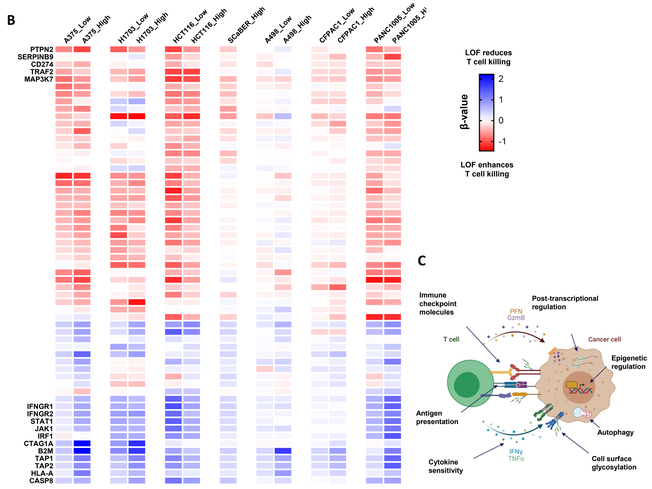


Figure 3: Summary of screen results from human cell line panel. (A) Example of a genome-wide screen in A375 cell line highlighting select genes that upon knockout, lead to sensitization or resistance to T cell killing. (B) Heatmap summarizing the top hits from profiling multiple human cancer cell lines. (C) Summary of key pathways controlling tumor killing by CD8 T cells.

IN VITRO TARGET VALIDATION

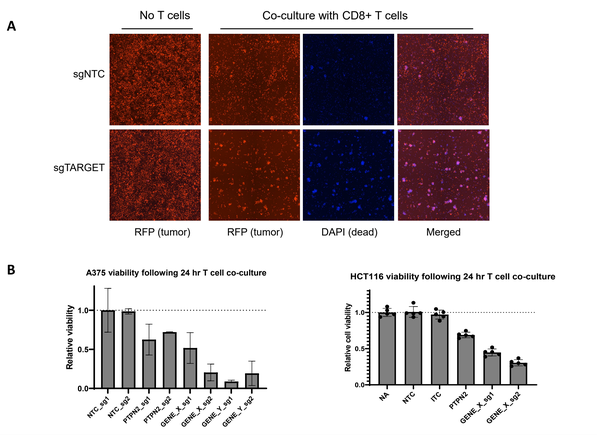


Figure 4: Microscopy assay for in vitro validation of co-culture hits. (A) Celigo based assay showing typical example of the observed phenotype for knockout of a sensitizing gene. (B) Genetic validation of PTPN2 and additional sensitizers using Celigo-based assay.

IN VIVO TARGET VALIDATION USING ACT MODEL

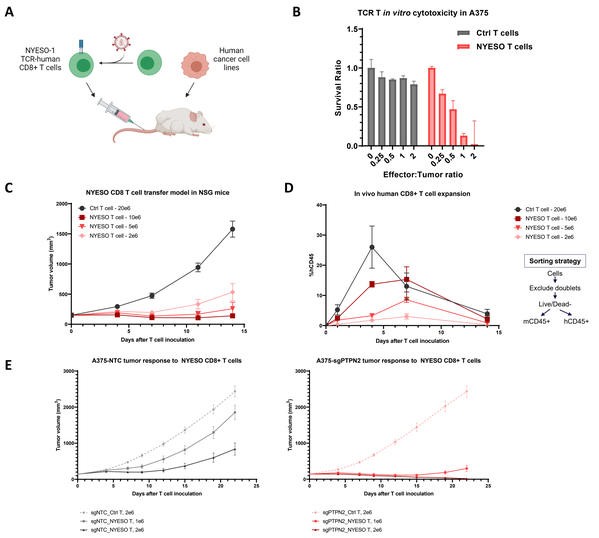


Figure 5: Human A375 cell line xenograft model with adoptive transfer of NYESO TCR+ CD8 T cells for target validation. (A) Schematic diagram of adoptive cell transfer model. (B) In vitro confirmation of antigen-specific killing of A375 melanoma cells by NYESO TCR+ CD8 T cells. (C) In vivo dose finding experiment showing dose-dependent tumor growth inhibition following infusion of NYESO TCR+ CD8 T cells. (D) Quantification of CD8+ T cell expansion from panel (C). (E) Measuring the effect of PTPN2 knockout by CRISPR/Cas9 RNP electroporation prior to cell inoculation compared to a non-target control sgRNA.

CONCLUSION/SUMMARY

- An in-vitro screening platform was established for screening human cancer genes that mediate sensitization and resistance to antigen-specific CD8 T cell killing
- Cell lines were selected based on intact MHC-I expression and HLA-A02:01 haplotype.
- Screen was performed in 7 cancer cell lines, highlighting key pathways involved in regulating killing of human cancer lines by antigen-specific CD8 T cells.
- Candidate genes were validated using in vitro and in vivo systems, showcased using the known sensitizer PTPN2

REFERENCES

1. Patel, S. J. *et al.* Identification of essential genes for cancer immunotherapy. *Nature* **548**, 537–542 (2017).
2. Manguso, R. T. *et al.* In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nature* **547**, 413–418 (2017).

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