283 GENOME-WIDE CRISPR SCREENS OF CYTOTOXIC T CELLS IDENTIFY A NOVEL REGULATOR THAT ENHANCES T CELL EFFECTOR FUNCTION

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Background Despite the immense therapeutic potential of cytotoxic T lymphocytes (CTLs) in anti-tumor immune response, it remains under-exploited given the limited knowledge we have on genes that regulate its function. Current immunotherapies modulating CTL functions are promising anti-tumor treatment options, but they are severely underutilized due to the lack of druggable targets and potential side effects associated with the treatments. Moreover, the development of new immunotherapies is highly driven by our improved understanding of genetic programs that influences T cell function. Therefore, identifying new immunotherapeutic gene targets is crucial to open new avenues for immunotherapies.

Methods In this study, we first performed an *in vitro* CRISPR-based genome-wide screen for negative regulators of CD8 T cell effector function by sorting for transduced cells with an increased expression of CD107a and tumor necrosis factor (TNF) after gene editing. Using next-generation sequencing, enriched genes in the respective populations were identified and ranked using the available PinAPL-Py platform.[1] To validate and characterize hundreds of the top ranked hits from genome-wide screen at greater details, we created a custom sgRNA mini-pool with newly designed sgRNA sequences for the screened hits. We repeated the screens with the mini-pool against various cancer cell lines as antigen presenting cells to validate the gene targets as well as *in vivo* mice tumour models to examine its effect on tumour infiltration.

Results Our screens robustly identified a gene target which encodes for an actin-binding domain and signal-mediator scaffolding protein that is important in regulating CTL's cytotoxic function. Preliminary data suggests that knocking out the gene target in mouse CD8 T cells showed increased degranulation and production of effector cytokines. Gene-KO T cells also exhibited greater killing efficacy against mouse B16 melanoma cells at various effector to target ratios compared to the control group transduced with non-targeting sgRNA. Further mechanistic studies and validation in mouse tumor models are currently in progress to support the findings.

Conclusions Overall, our data demonstrate that genetic screens for immunotherapeutic gene target discovery is essential to identify new regulators of CTLs. We also showed that our identified gene target plays an important role in modulating the effector function of CTLs and suggests that manipulation of the gene would improve cancer immunotherapy.

REFERENCE

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