In vivo CRISPR screen identifies tumor suppressors as drivers of tumor cell-intrinsic immune evasion

ABSTRACT# 1905

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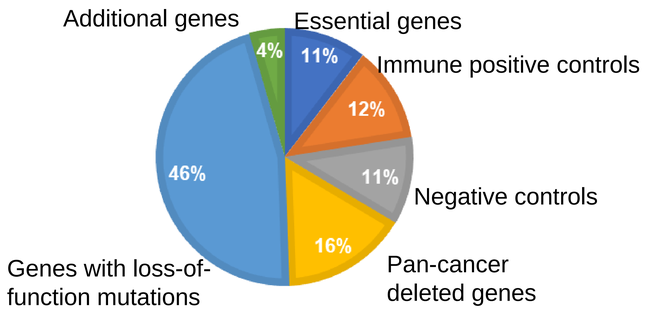
ABSTRACT

Cancer cell-intrinsic genetic alterations that allow cancer cells to evade destruction by the immune system remain poorly characterized. Here we performed a pooled CRISPR-Cas9-based *in vivo* genetic screen targeted to a pre-defined set of tumor suppressor genes to mimic loss-of-function mutations in syngeneic tumor models. CRISPR edited tumor cells were implanted into immune-deficient or immune-competent C57BL/6 mice, a subset of which were treated with anti-PD1 to simulate increased immune pressure. Tumor samples at the endpoint were subjected to next generation sequencing analysis to identify tumor suppressor genes driving immune evasion. The screen confirmed previously identified immunotherapy targets such as CD47 and Adar as well as known drivers of immune resistance in the interferon signaling and antigen presentation pathways. Importantly, we identified loss of STK11 and KEAP1 as drivers of immune resistance in syngeneic models. TIL profiling analysis suggests that STK11 knockout induces a “cold” tumor microenvironment. *STK11* and/or *KEAP1* genomic alterations are found in ~25% of non-squamous non-small cell lung cancer and have been reported as a major predictor of primary resistance to PD-1 blockade. Together, these data demonstrate that high-throughput *in vivo* genetic screens can identify tumor cell-intrinsic drivers of immune evasion and establish murine system relevant to the study of primary resistance to PD-1 axis immunotherapy and more generally, tumor cell-intrinsic immune evasion.

CRISPR tumor suppressor library



n = 162 genes; 10sgRNAs/gene

In vivo screen design

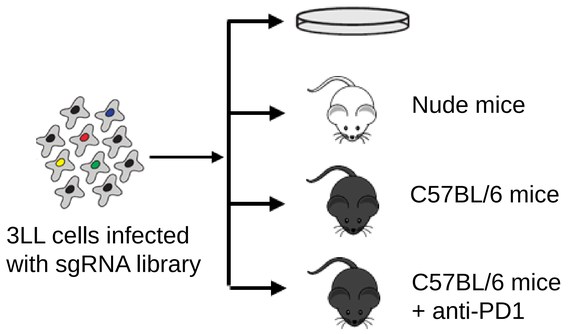


Figure 2: Diagram of in vivo screen platform and design. 3LL cells were transduced with CRISPR tumor suppressor library and stable cell line generated by puromycin selection. Stable cells were inoculated into nude mice, C57BL/6 mice and C57BL/6 mice treated with anti-PD1 representing increasing degrees of anti-tumor immunity.

Tumor growth reflects increased anti-tumor immunity *in vivo*

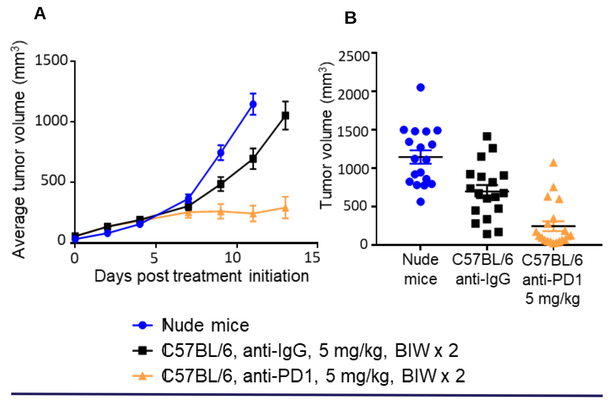


Figure 3: Tumors grow slower with increasing immune pressure in vivo. Mice were grouped on day 0 when average tumor volumes reach 50mm^3. Average tumor volumes for each condition were plotted. Gradually decreasing tumor growth from nude mice to C57BL/6 treated with anti-IgG to C57BL/6 mice treated with anti-PD1 reflects increased anti-tumor immunity in these three in vivo conditions.

Positive controls work well in the screen

Figure 4: Positive control genes were depleted as expected. sgRNAs for Plk1, an essential gene, were depleted in all conditions relative to plasmid pool. sgRNAs targeting Cd47, a well-known immune target for macrophages, were depleted as expected in all in vivo conditions.

STK11 and KEAP1 are among the top resistant genes to immune pressure

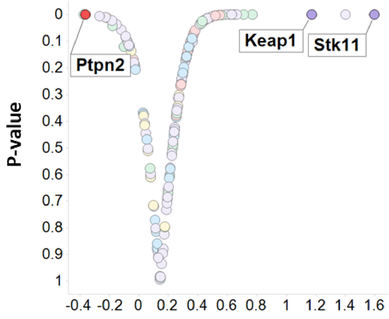


Figure 5: STK11 and KEAP1 are among the top resistant genes from the screen. sgRNA depletion or enrichment from tumors of C57BL/6 mice + anti-PD1 vs. C57BL/6 mice + anti-IgG is represented in a volcano plot. sgRNAs for Stk11 and Keap1 are the top two most enriched in C57BL/6 treated with anti-PD1 vs anti-IgG whereas sgRNAs for Ptpn2 were among the most depleted.

STK11 knockout drives immune evasion

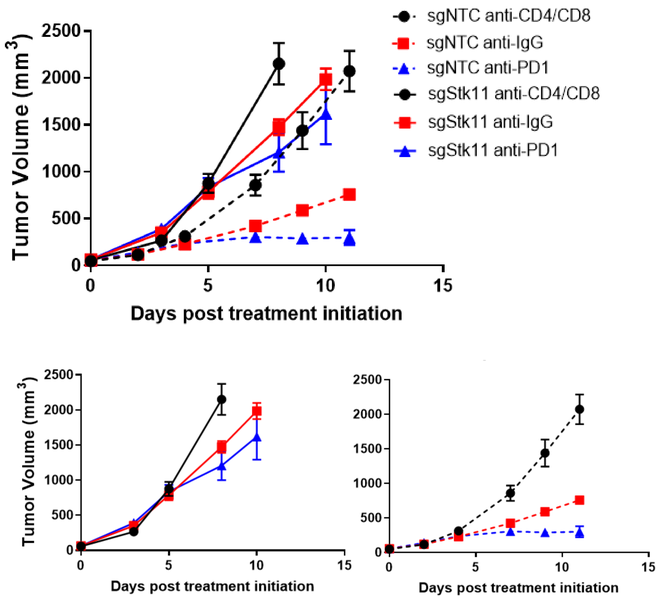


Figure 6: STK11 knockout drives resistance to immune pressure in vivo. 3LL cells were transduced with sgRNAs targeting STK11 or non-targeting control (NTC). Derivatives were inoculated into C57BL6 mice treated with anti-PD1 or anti-IgG2a or anti-CD4/CD8 to deplete T cells. Depletion of STK11 drives resistance to increasing immune pressure in vivo.

KEAP1 knockout drives immune evasion

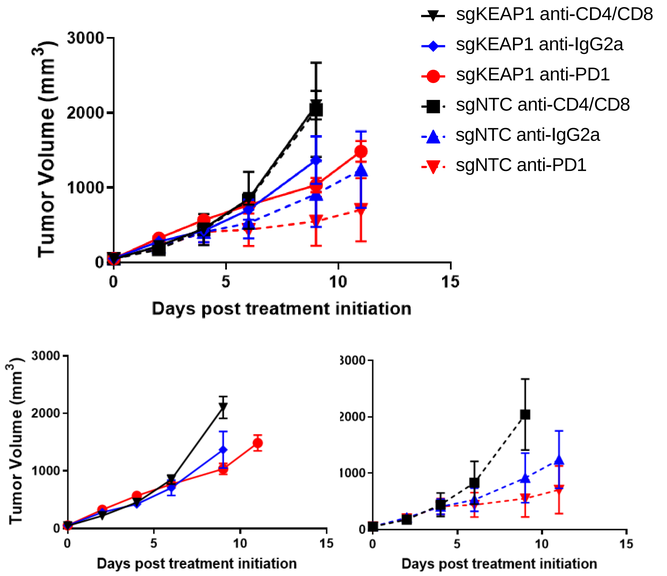


Figure 7: KEAP1 knockout drives resistance to immune pressure in vivo. 3LL cells were transduced with sgRNAs targeting KEAP1 or non-targeting control (NTC). Derivatives were inoculated into C57BL6 mice treated with anti-PD1 or anti-IgG2a or anti-CD4/CD8 to deplete T cells. Depletion of KEAP1 drives resistance to increasing immune pressure in vivo.

STK11 loss drives a cold and suppressive immune environment

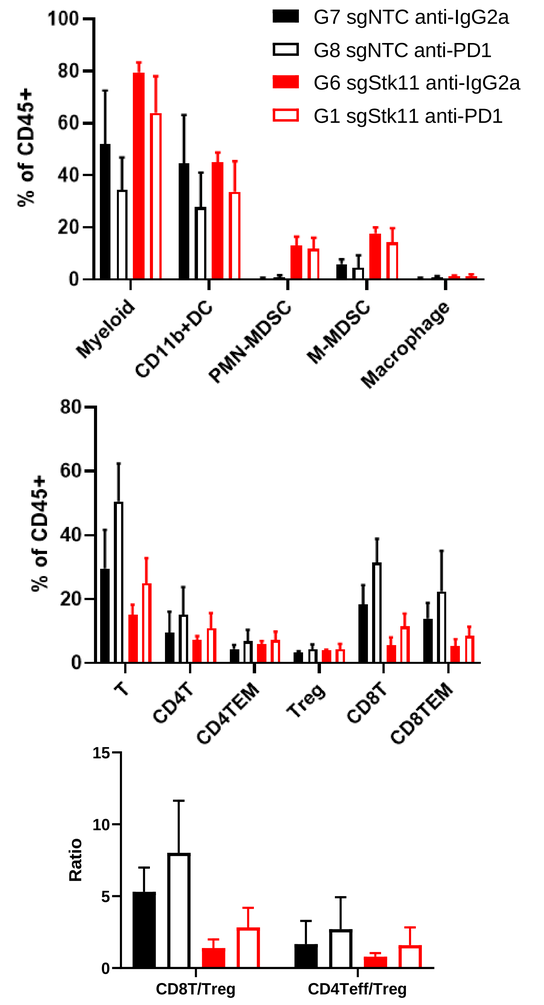


Figure 8: STK11 knockout drives a suppressive immune environment in vivo. Immunophenotyping analysis were conducted on sgNTC vs sgStk11 tumors upon anti-PD1 or anti-IgG2a treatment. Stk11 KO increased the frequency of MDSCs, decreased CD3 T cells and CD8 T cells and CD8TEM (effector memory T cells) in the tumor immune environment.

SUMMARY

- A pooled CRISPR-Cas9-based in vivo screen was performed successfully in conditions reflecting increased anti-tumor immunity
- STK11 and KEAP1 were identified as immune evasion contexts
- Depletion of STK11 or KEAP1 drives resistant to immune pressure in immune competent mice
- Immunophenotyping analysis comparing STK11 knockout tumors vs control indicated STK11 loss drives a cold and suppressive immune environment