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Tumor evolution inactivates microRNA machinery for immune evasion

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Abstract

BACKGROUND: Cancer arises after somatic mutations override cellular and immunological control of cell proliferation. Mutations in oncogenes and tumor suppressor genes endow cancer cells intrinsic growth advantage over neighboring cells. Mutations can also enable cancer cells to escape from immune surveillance during cancer evolution, but a targeted approach to uncover such mutations is lacking.

RESULTS: We hypothesized that genetic heterogeneity intrinsic to cancer cells affects the cell fitness to T cell-mediated cytotoxicity. By deep sequencing of murine tumors grown under different conditions of immune editing, we identified 59 genetic mutations in clonal populations that were specifically enriched under host immune pressure. To differentiate driver from passenger mutations against immune selection, we performed in vivo and T cell co-culture CRISPR screens with a guide RNA library targeting the corresponding genes. We identified ANKRD52 as required for PD-1-independent T cell-mediated cytotoxicity, which enhanced the JAK-STAT-interferon-γ signaling and antigen presentation in cancer cells. ANKRD52, in complex with PPP6C, dephosphorylates AGO2 and promoted microRNA (miRNA)-targeted silencing of suppressor of cytokine signaling 1 (SOCS1), and deletion of SOCS1 reinstalled the sensitivity of the mutant cells to T cell killing. Introduction of patient hotspot ANKRD52 mutations, or deletion of AGO2, DICER1 or XPO5 in cancer cells compromised the interferon-γ and T cell response.

SUMMARY: Here we combined immune pressure selection and CRISPR screen validation in vivo to identify spontaneous or rare mutations in cancer cells that endow resistance to T cell-mediated cytotoxicity. Our data indicate that the evolutionarily conserved miRNA pathway is exploited by cancer cells for immune escape.

Fig1. Profiling of cancer heterogeneity selected by host immunity

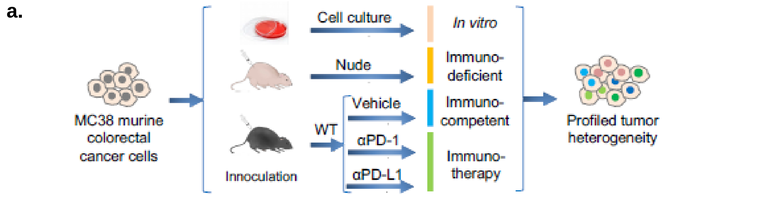

Fig. 1| Mutation profiling of cell line derived xenograft (CDX) tumors under progressive immune selection. a, Schematic of MC38 CDX tumors grown in host mice harboring different levels of T cell immunity.

Fig2. Identification of ANKRD52 as a modulator of T cell immunity

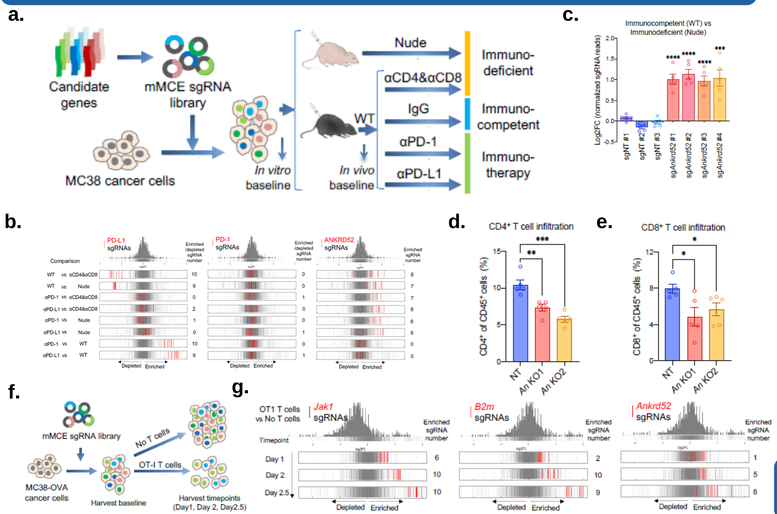

Fig. 2| Identification of ANKRD52 as a modulator of T cell immunity by targeted in vivo and co-culture CRISPR library screens. a, Schematic of in vivo CRISPR screen to validate candidates from immune-selected mutations. b, Distribution histograms of enrichment or depletion (log2FC) for all 10 sgRNAs targeting PD-1, PD-L1 or ANKRD52 as indicated as red lines, overlaid on gray gradient depicting the overall sgRNA distribution. |FC| > 1.5, P < 0.05. c, In vivo competition assay with equal number mixture of MC38 cells infected with sgRNAs for non-targeting control (NT) or Ankrd52 in WT (n = 5) or nude mice (n = 5). d and e, Flow cytometry analysis of CD4+ (d) and CD8+ (e) T cell populations from NT and Ankrd52 knock-out (An KO) tumors (n = 5 per group). f, Schematic of OT-I T cell co-culture CRISPR screen with MC38-OVA cells. g, Distribution histograms of enrichment for all 10 sgRNAs targeting Jak1, B2m or Ankrd52. FC > 1.4, P < 0.05.

Fig3. Attenuated IFNγ response by ANKRD52 mutations

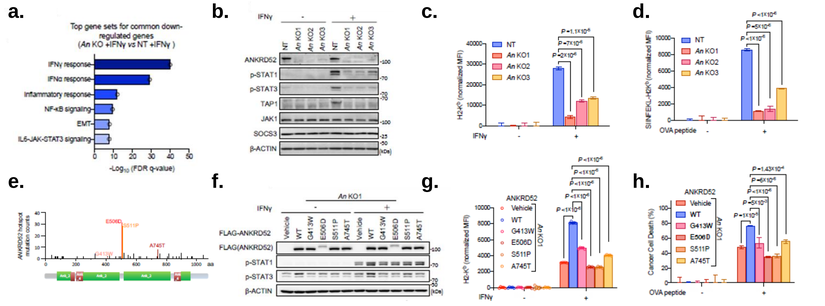

Fig. 3| Impaired IFNγ response and antigen presentation by ANKRD52 inactivation. a, Hallmark gene sets enriched for commonly down-regulated genes in two ANKRD52 KO MC38 clones compared to control cells after IFNγ treatment. b,c, Abundance of IFNγ signaling proteins (b) and membrane MHC-I expression (c) in control and Ankrd52-null MC38 cells treated with IFNγ d, SIINFEKL-H2Kb presentation in OVA-treated control and Ankrd52-null MC38 cells. e, Localization of clinical hotspot ANKRD52 mutations from combined TCGA, COSMIC and OncoWuxi databases. f,g, Protein abundance of p-STAT1 and p-STAT3 (f) and membrane MHC-I expression (g) in Ankrd52-null MC38 cells expressing WT or mutant ANKRD52 after treatment. h, Killing of OVA-treated Ankrd52-null MC38 cells expressing WT or indicated mutant ANKRD52 by OT-I T cells.

Fig4. Regulation of cancer immunity by miRNA machinery

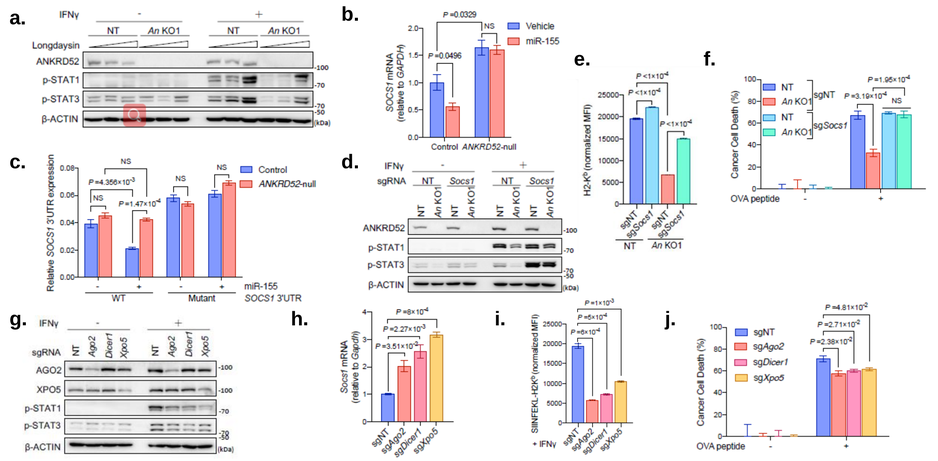

Fig. 4| Requirement of the miRNA biogenesis and targeting machinery for cancer cell response to T cell-mediated killing. a, p-STAT1 and p-STAT3 abundance in MC38 cells treated with IFNγ and increasing Longdaysin (0, 50, 100μM). b, SOCS1 mRNA level in 293T cells overexpressing miR-155. c, Activity of WT and mutant SOCS1 3'UTR in a dual luciferase reporter in 293T cells overexpressing miR-155. d,e, p-STAT1 and p-STAT3 abundance (d) and membrane MHC-1 expression (e) in Ankrd52-null MC38 cells with inactivated Socs1. f, Killing of OVA-treated Ankrd52-null MC38 cells with inactivated Socs1 by OT-I T cells. g,h, p-STAT1 and p-STAT3 abundance (g) and SOCS1 mRNA level (h) in MC38 cells with targeted deletion of Ago2, Dicer1 or Xpo5 after stimulation with IFNγ. i, SIINFEKL-H2Kb presentation in OVA-treated MC38 cells with targeted deletions. j, Killing of OVA-treated mutant MC38 cells by OT-I T cells.

SUMMARY

- Cancer heterogeneity selected by host immunity was profiled for key immune escaping mutation calling.
- *In vivo* and *in vitro* CRISPR screen validation were used to identify spontaneous or rare mutations in cancer cells that endow resistance to T cell-mediated cytotoxicity.
- Our studies suggest patients with defective miRNA biogenesis may not respond well to T cell-based immunotherapies..

Collaboration with industry and academia is welcome.