Identifying lncRNA modulators of T-cell - Tumor cells interaction to enhance immune checkpoint inhibition therapies

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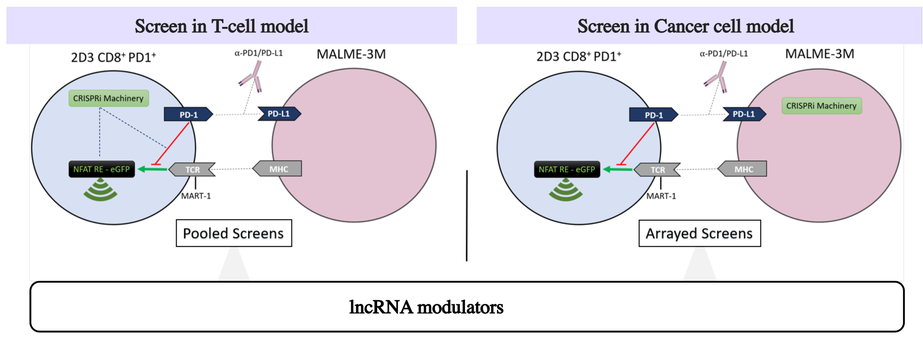
INTRODUCTION

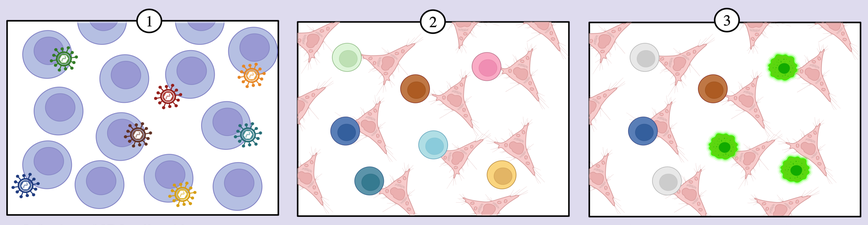
We aim to identify immuno-modulating lncRNAs in both T-cells and tumor cells that could enhance immune checkpoint inhibition. The discovery and further characterization of candidates will be performed using CRISPR interference (CRISPRi) pooled and arrayed screening platforms applied to a dedicated T-cell tumor cell co-culture system.

This system is based on a Jurkat derived cell line (2D3), which expresses an NFAT eGFP reporter gene reflecting TCR signaling. These cells were stably transduced with a MART1 TCR and respond to MART1 antigen presentation when co-cultured with melanoma cells. Both our tumor and T-cell models stably express dead Cas9 (dCas9) fused with the transcriptional repressor KRAB-MeCP2 for CRISPR interference applications.



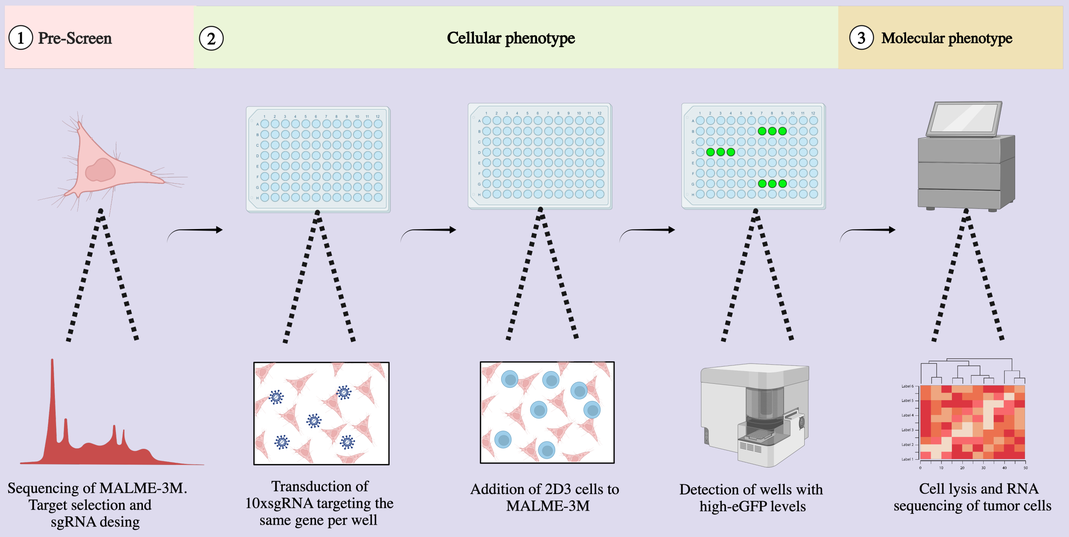
METHODS

2D3: Pooled screen



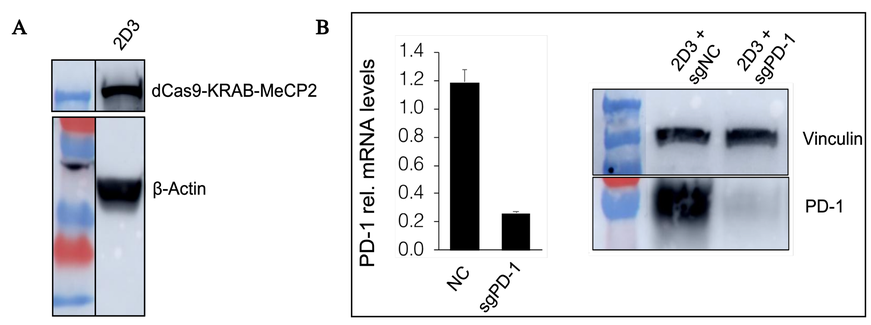
1. Infection of 2D3 cells with a custom sgRNA library against lincRNA
2. T_0 **DNAseq** to stablish sgRNA baseline and addition of transduced 2D3 cells to previously seeded MALME-3M cells
3. 24hs after sgRNA transduction, lincRNA that repress immune activation will be enriched in the eGFP positive population
4. eGFP based cell sorting of 2D3 cells
5. T_F **DNAseq** to stablish HITs of the screen

MALME-3M: Arrayed screen workflow



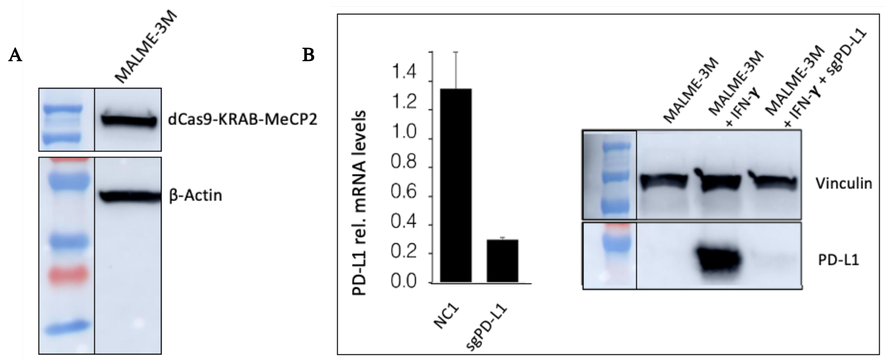
RESULTS

2D3 dCAS-KRAB-MeCP2 Validation: PD-1 mRNA and protein levels



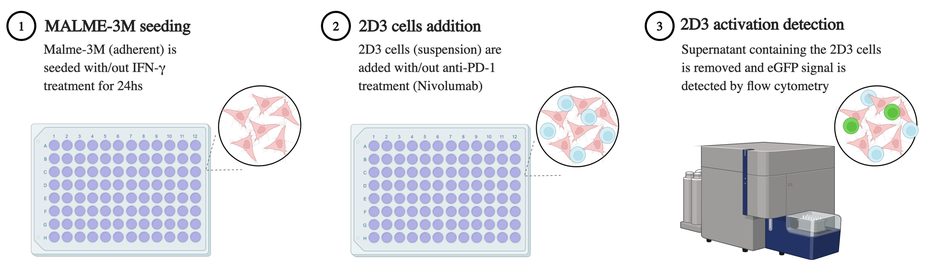
(A) Western-Blot of 2D3 cells transduced with dCas9-KRAB-MeCP2 machinery. (B) 2D3 cells transduced with a sgRNA against PD-1. Verification of mRNA and protein levels by qPCR and Western-Blot. Negative control (NC) is a sgRNA unable to target any region of the human genome.

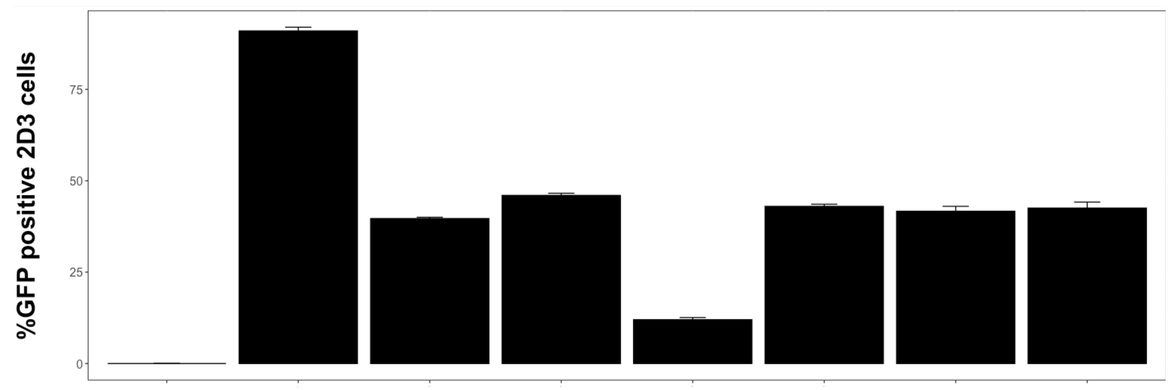
MALME-3M dCAS-KRAB-MeCP2 Validation: PD-L1 mRNA and protein levels



(A) Western-Blot of MALME-3M cells transduced with dCas9-KRAB-MeCP2 machinery. (B) Transduction of 2D3 cells with a sgRNA against PD-L1. Verification of mRNA and protein levels by qPCR and Western-Blot. Negative control (NC) is a sgRNA unable to target any region of the human genome. Treatment of MALME-3M with interferon gamma (IFN-γ) leads to PD-L1 upregulation.

Validation of co-culture model system





2D3	+	+	+	+	+	+	+	+
MALME-3M	-	-	+	+	+	+	+	+
PMA/ION	-	+	-	-	-	-	-	-
NIVOLUMAB	-	-	-	+	-	+	-	-
IFN-γ	-	-	-	-	-	+	+	+
sgPD-1	-	-	-	-	+	-	+	-
sgPD-L1	-	-	-	-	-	-	-	+

*Phorbol myristate acetate (PMA) and Ionomycin (ION) can bypass the T cell membrane receptor complex (TCR) and in combination lead to the upregulation of several intracellular signalling pathways such as NFAT, resulting in T cell activation. They constitute our positive control.

CONCLUSION

We successfully optimized a model to investigate lincRNA modulators of T-cell activation. Pre-treatment of MALME-3M with IFN-γ in co-culture with 2D3 cells leads to reduced TCR-signaling and consequently lower eGFP levels. Knocking down negative modulators such as PD-1 (2D3) or PD-L1 (MALME-3M) restores eGFP to baseline/Nivolumab-treated levels. These results validate our model systems and suggests that negative modulators of TCR-signaling can be revealed using CRISPR interference. Candidate lincRNAs will be identified using both pooled and arrayed screens in the 2D3 cells and tumor cells respectively.

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