5-2022

Development Of Whole-Genome CRISPR-CAS9 Screens To Discover Novel Immunotherapy Targets In Pediatric Leukaemia

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DEVELOPMENT OF WHOLE-GENOME CRISPR-CAS9 SCREENS TO DISCOVER NOVEL IMMUNOTHERAPY TARGETS IN PEDIATRIC LEUKAEMIA

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BACKGROUND & RESEARCH QUESTION

Therapy-resistant cells are responsible for cancer treatment failure, but targeting these cells remains challenging. Minimal residual disease (MRD) contains chemoresistant leukemia stem cells (LSCs), which are particularly resistant to therapy and reduce the risk of relapse.

Treatment of pediatric leukaemia often involves the use of high doses of anthracyclines, which results in severe side-effects. Based on previous studies showing that anthracyclines such as doxorubicin (DXR) can target therapy-resistant leukemia stem cells (LSCs) at low, non-toxic doses, we repurposed DXR as a targeted inhibitor rather than a broadly cytotoxic chemotherapy. Unlike clinical usage, low-dose DXR treatment reduces expression of multiple immune checkpoint (IC) genes, which exposes LSCs to elimination by cytotoxic T lymphocytes.

Here, we use whole genome CRISPR-Cas9 screening to investigate the mechanisms that distinguish immunotherapeutic vs chemotherapeutic doses of anthracyclines in pediatric leukaemia.

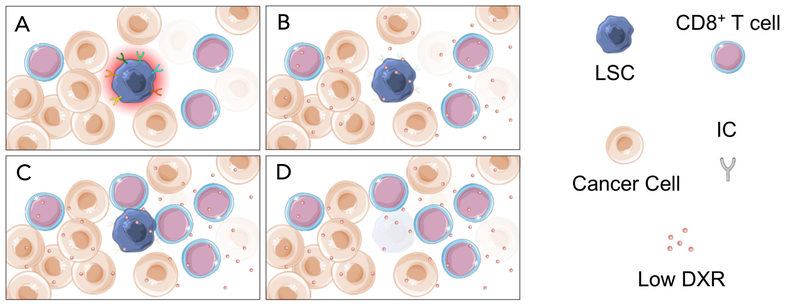

Figure 1. Therapy resistant LSCs exhibit unique properties of immune escape that can be targeted. (A) LSCs express diverse immune checkpoints, which protects from elimination by T cells. (B) Low dose DXR inhibits IC expression. (C-D) Now lacking their immuno-suppressive shield, LSCs are exposed to anti-cancer T cell activity and selectively targeted (Perry et al., 2020. Nat Cell Bio).

DESIGN

We are currently utilizing CRISPR-Cas9 gene editing technology to construct and validate a chemical-genetic strategy for identifying immune checkpoint (IC) suppressors and immunogenic cell death (ICD) inducers. We are optimizing combined genome-wide repression (using CRISPRi) and overexpression (using CRISPRa) screening systems on pediatric leukemia cells treated with low to high exposures of anthracyclines to discover genes that antagonize and synergize with IC suppression/ICD induction vs. drug resistance.

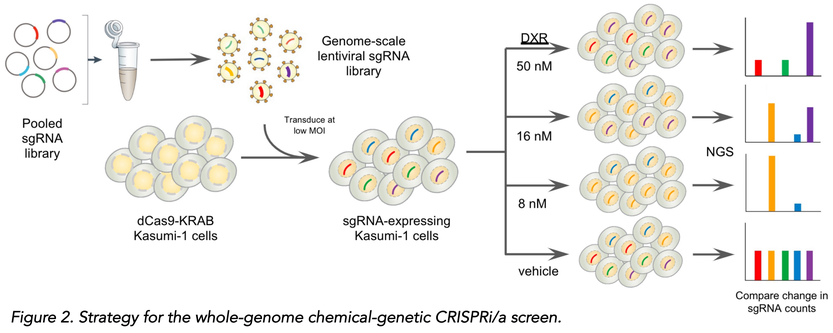

Figure 2. Strategy for the whole-genome chemical-genetic CRISPRi/a screen.

DEVELOPMENT & VALIDATION

Preparation of sgRNA Libraries

CRISPRi & CRISPRa whole genome libraries have been amplified, sequenced, and packaged into lentiviral particles. Each library contains > 18,000 human genes and each gene is targeted by 3 unique sgRNAs.

Generation of dCas9-stable Cell Lines & Validation of dCas9 Function

Leukaemia cell lines are being designed to stably express deactived-Cas9 (dCas9) or dCas9-KRAB (Fig. 3A) to activate or inhibit transcription, respectively, when transduced with the sgRNA libraries. To determine if the newly established dCas9- and dCas9-KRAB- expressing cell lines are active, introduction of lentivirus for positive control sgRNAs targeting human cell surface genes that have shown high activity have been tested. These sgRNA constructs are infected into each corresponding CRISPRi or CRISPRa line and knockdown or overexpression efficiency is measured by flow cytometry (Fig. 3B).

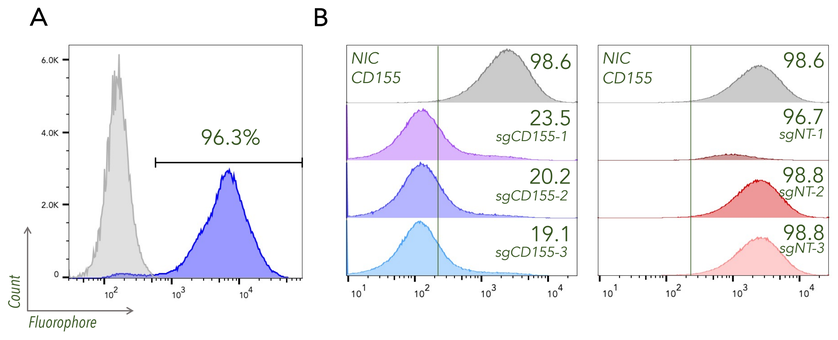

Figure 3. (A) Generation of dCas9-stable cell lines. After transduction of dCas9 lentiviral particles, leukemia cells were FACS sorted for selection of BFP-positive dCas9. (B) Validation of dCas9 function. Nearly all dCas9-stable leukemia cells express CD155 on their cell surface, however, when transduced with each of the 3 sgRNA lentivirus against CD155 expression is knocked-down. This experiment confirms that dCas9 is indeed functional in the stable cell line. NIC = non-infected control.

Determining Immunogenic Doses of DXR

To determine the appropriate exposure scenarios for our human stable cell lines, with the goal of recapitulating the biological effects of the clinical and targeted doses of DXR as demonstrated in our mouse study (Nat Cell Bio. 2020), we analyzed a range of DXR doses in a timecourse for the expression of multiple IC inhibitors and the ICD inducer calreticulin (CALR).

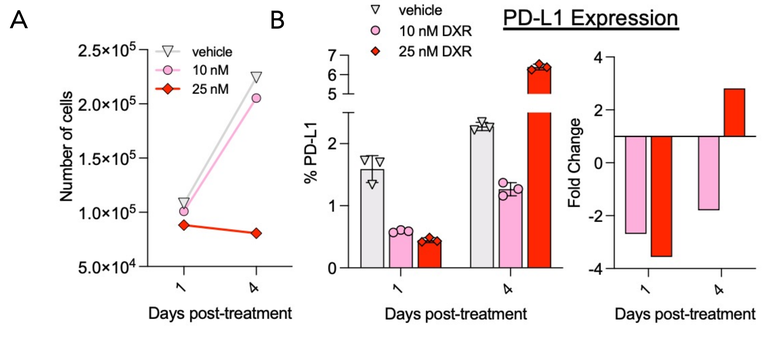

Figure 4. Inhibition of immune checkpoints by low/targeted anthracycline treatment in patient-derived leukemia cells. (A) Cell proliferation with differential exposure to DXR. (B) % PD-L1+ Kasumi-1 cells and fold change (vs vehicle) in PD-L1 expression at low vs high DXR exposure. This is the same effect as seen in mouse LSCs from our previous studies indicating that we are able to recapitulate the targeted DXR mechanism for this study.

IC inhibitors prevent the immune system from overacting. Leukaemia stem cells (LSCs) use this to their advantage to protect themselves from immune attack. In contrast to typical clinical usage, low-dose DXR treatment reduces expression of multiple IC inhibitors, which exposes LSCs to elimination by cytotoxic T lymphocytes. We have been able to capture this same effect, of targeted DXR exposure (low, sustained dose) and clinical DXR exposure (high, cytotoxic dose), so that we can apply and recapitulate that same immunogenic function in our dCas9-stable leukaemia cell lines for the whole-genome screen (Fig 4).

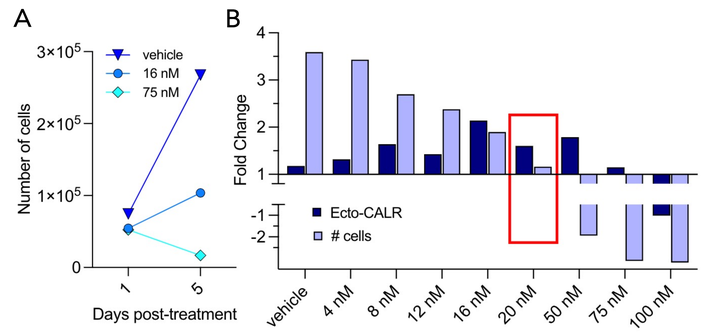

Figure 5. "Intermediate" exposure range inducing ICD. (A) Leukaemia cell proliferation with differential exposure to DXR. (B) Fold change of ecto-CALR expression and cell numbers from day 1 to day 5. Red box indicates exposure where cell expansion stops and ecto-CALR is induced.

In the laboratory, anthracyclines can also stimulate ICD. Unlike other forms of cellular death, ICD can induce an anti-cancer immune response, effectively immunizing against cancer recurrence. As the first responder to cell-stress (and potentially ICD induction), we monitored CALR expression. Unexpectedly, we found what appears to be a possible "intermediate" exposure range where CALR is indicating cellular stress, yet the live cells population is well enough to continue proliferating (16 nM DXR)(Fig 7).

ANALYSIS of GENE HITS

Currently, we are analyzing the sequencing data from the first screen and identifying the top positively- and negatively-selected gene hits. The selected biologically relevant hits will be further validated and then studied in depth for potential therapeutic uses.

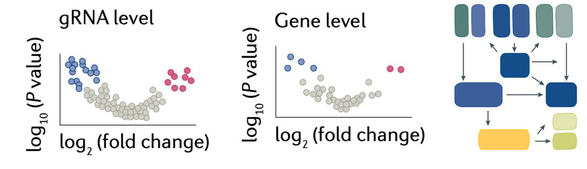

Figure 6. Graphic representation of gene ranking & hit analysis. Figure from Bock et al., 2022. Nat Rev Methods Primers.

Ultimately, this project will reveal novel therapeutic strategies for harnessing the immune system to enhance and potentiate the ICD response of low-dose DXR treatment, thus providing durable, less toxic cures for pediatric cancer.

ACKNOWLEDGEMENTS

Braden's HOPE for Childhood Cancer
NOAH'S BANDAGE PROJECT
STOWERS INSTITUTE FOR MEDICAL RESEARCH
UMKC School of Medicine
KU SCHOOL OF MEDICINE The University of Kansas
Children's Mercy KANSAS CITY | Research Institute