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TITLE:	Single-Cell CRISPRa Screen to Identify Transcription Factors That Mediate Neuroblastoma Phenotypic Switching and Chemotherapy Drug Resistance
PRINCIPAL INVESTIGATOR:	Dr. Zhihui Liu
CONTRACTING ORGANIZATION:	The Geneva Foundation, Tacoma, WA
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Objectives: I hypothesize that there are other ADRN-and MES-specific TFs that can mediate trans-differentiation and in extension chemotherapy resistance. Furthermore, I propose that the combinatorial cooperation of several TFs will be more potent than a single TF to facilitate this process. To test my hypothesis, I propose two specific aims: 1) aim 1 is to identify TFs and TF combinations that regulate NB trans-differentiation; 2) aim 2 is to identify TFs and TF combinations that contribute to chemotherapy resistance of NB.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

Neuroblastoma (NB) is heterogenous, and it mainly includes adrenergic (ADRN) and mesenchymal (MES) tumor cell types. These NB subtypes are able to trans-differentiate or interconvert. The phenotypic and genotypic plasticity most likely contributes to the fact that many high-risk tumors initially respond to chemotherapy drugs but ultimately relapse. In this proposal, I will adapt recently developed single-cell CRISPRa sequencing technique to evaluate a novel concept of one or a group of TFs are essential in regulating NB cell phenotypic switching and chemotherapy drug resistance in NB

2. KEYWORDS:

Neuroblastoma, transcription factors, core regulatory circuitry, phenotypic switching, CRISPRa, single-cell RNA sequencing, CRISP-seq, perturb-seq

3. ACCOMPLISHMENTS:

What were the major goals of the project?

There are two major goals of this project: 1. Identify transcription factors (TFs) and TF combinations that regulate neuroblastoma (NB) cells trans-differentiation; 2. Identify TFs and TF combinations that contribute to chemotherapy resistance of NB.

What was accomplished under these goals?

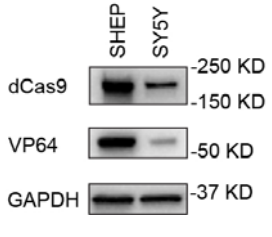

Fig. 1. Verification NB-CRISPRa clones

Aim 1. Identify transcription factors (TFs) and TF combinations that regulate neuroblastoma (NB) cells trans-differentiation.

Major Task 1. *Generating NB stable cell lines with the CRISPR system.* By transducing dCas9-SunTag and scFv-VP64 lentiviral particles into SY5Y and SHEP cells, and selecting the cell with blasticidin and geneticin, I have established stable SY5Y-CRISPRa cell line and SHEP-CRISPRa cell line. The expression of dCas9 and VP64 was detected by western blot assay (Fig. 1). Major Task 1 has been accomplished.

Major Task 2. *Generating sgRNA libraries.* I have designed two

sgRNA libraries to target specific group of TFs. The special sgRNAs have been successfully cloned into dual-guide 3' direct-capture Perturb-seq vectors and verified by DNA sequencing. I have also generated lentiviral particles for these two sgRNA libraries. Major Task 2 has been accomplished.

Major Task 3. *Perform CRISPRa-seq to identify TFs that regulate NB cells phenotypic switching.* This task has not been started. I have tried to generate SHEP-CRISPRa and SY5Y-CRISPRa stable clones using different methods, different vector systems, but I have observed phenotypic switching of these two cell lines during single clone select. After several months trouble shooting, I just got the SHEP-CRISPRa and SY5Y-CRISPRa stable cell lines that express both dCas9 and VP64 recently. To further confirm the CRISPRa system and the established stable NB cell lines could work the way as expected, I plan to validate the system before performing single-cell CRISPRa sequencing. We have cloned HAND2 and NOTCH2 sgRNAs into the dual-guide 3' direct-capture Perturb-seq vector. I will investigate whether the transfection of sgHAND2 vector into SHEP-CRISPRa cells could activate HAND2 expression, and wether the transfection of sgNOTCH2 vector into SY5Y-CRISPRa cells could activate NOTCH2 expression. These experiments are ongoing.

Specific Aim 2. Identify TFs and TF combinations that contribute to chemotherapy resistance of NB.

This task has not been started. I will start this task as soon as I finish the specific aim 1, major task 3.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

Currently, I am validating our CRISPRa and dual-guide vector system in SY5Y-CRISPRa cell line and SHEP-CRISPRa cell line. As soon as this is done, I will start major task 3, which is to perform CRISPRa-seq to identify TFs that regulate NB cells phenotypic switching by infecting these cell lines with the sgRNA libraries I generated. Next I will start major task 4, which is to perform CRISPRa-seq to identify TFs that protect NB cells from chemotherapy drugs.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

Nothing to report.

  Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

PI: Zhihui Liu
No change.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.