Developing a Genetic Screen for Identification of Genes Involved in Chemoresistance For Cancer Therapy Using DNA Barcoding

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Abstract

Resistance to chemotherapy is one of the biggest hurdles in cancer therapy, hindered further by our limited knowledge of the molecular mechanisms of chemoresistance. We aim to identify the genes involved in chemoresistance using a functional genomics approach by creating a genetic screen that utilizes DNA barcoding. We established normal cell lines and transduced sgRNA into them; then, we exposed the target cells to standard chemotherapy and sequence the sgRNAs that expressed the phenotype of chemotherapy resistance using Sanger sequencing. We were able to use DNA barcoding to identify genes that were resistant to chemotherapy and create an effective genetic screen.

Introduction

- Chemotherapy only treatment for cancers without targeted therapies
- Chemoresistance is the biggest hurdle in cancer therapy, largely because of our lack of knowledge about the molecular mechanisms of chemoresistance
- Combination therapy cannot be standardized, every patient is different
- No comprehensive studies that catalog cancer alterations
- We aimed to assess how experimentally controlled alterations in every individual gene of the genome modify the response to chemotherapy
- By integrating these results with available mutagenesis data of human cancers we can identify genes involved in chemoresistance.
- Novel use of DNA barcoding to create a genetic screen

Materials and Methods

- Functional genomics approach: design, generation and validation of large plasmid libraries to perform loss-of-function studies
- sgRNA plasmid libraries were pooled and transduced into cells to to produce virus libraries
- These viruses were added to target cells, and these cells were subsequently exposed to anticancer drugs (paclitaxel and doxorubicin for mammary epithelial cells)
- We isolated 36 colonies, and performed Sanger sequencing
- To determine the alterations, sequences of the resistant colonies were aligned with each other and a sequence lacking resistance
- To identify the specific gene, we checked TCGA data sets, which contain information about thousands of human cancers

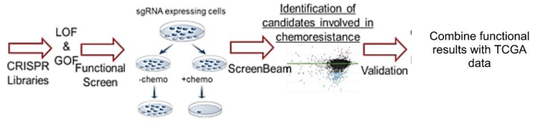
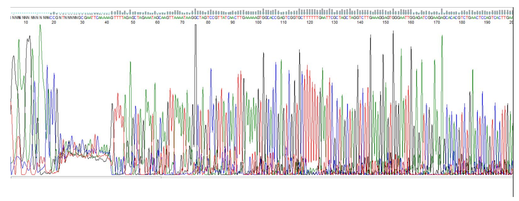


Image 1: Sanger sequencing

Results



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DEFINITION  2R-R_D01_ab1
ORIGIN
        1 NNNNNNANNG CNCCGACTCG GTGCCACTTG GCCCTGCAGA CATGGGTGAT CCTCATGTTG
       61 GCCAAGTTGA TAACGGACTA GCCTTATTTT AACTTGCTAG GCCCTGCAGA CATGGGTGAT
      121 CCTCATGTTG GCCTAGCTCT AAAACCCTTG CGGGAGAATA GATCG CGGTG TTTCGTCCTT
      181 TCCACAAAAT CCTCATGTTG GCCTAGCTCT AAAACGCCGG CAACCGAATT NATCGCGGTG
      241 TTTCGTCCTT TCCACAAAAN GNNN
//
```

Image 2: DNA Sequence Aligning

Gene ID	Frequency
SERHL2	1
CCDC154	1
C3orf55	1
ARMC8	1
C7orf69	1
POMT2	1
GP6	1
ZNF781	1
INTS7	1
C7orf31	1
SLC6A14	1
BAX	1
ZNF625	1

Image 3: Gene Library Matching

Discussion

- Using DNA barcoding to develop a genetic screen was successful
- Able to come up with a screen that could identify genes that manifested phenotypes we wanted
- Allows for identification of the genes most involved with chemoresistance
- Opens up treatment options, allows for more varied treatment for different kinds of cancers using knowledge of chemoresistance
- Allows for breakthroughs in diagnosis and treatment

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