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Breast Cancer Cell Migration and Engineering a CRISPRa System to Activate Dormant Tumor Suppressor Genes

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Summer Undergraduate Research in Biology

Abstract

CRISPR activation (CRISPRa) is a technology for gene transcription built on the discovery of programmable CRISPR endonucleases. While traditional CRISPR technology causes gene deletion and gene inhibition, CRISPRa is a lesser-explored system that has the ability to activate genes and thus reveal the function of dormant or lowly-expressed genes.[1] My research explores the utility of this novel strategy to identify a new repertoire of breast cancer targets. Specifically, my hypothesis is that CRISPRa can be optimized to prioritize tumor-actionable targets that I am calling "epigenetically dormant tumor suppressor" genes.[2]

My experimental approach was to a) characterize cancer cell phenotypes using live cell imaging and b) carry out molecular cloning to engineer an inducible CRISPRa system to test the role of candidate tumor suppressor genes. Cancer cell phenotypes were characterized by live cell imaging. Specifically, I tracked cell growth and cell motility. In parallel work, I cloned a gRNA gene for inducible CRISPRa of GAS1, a candidate tumor suppressor gene. Upcoming studies will test the causal relationship between GAS1 expression and cancer replication and motility.

Introduction

In its original form, CRISPR employs a Cas9 endonuclease, an enzyme that cleaves specific DNA segments by virtue of base-pair complementarity with a guide RNA (gRNA). The Cas9-gRNA complex can locate a specific section along the genome and, with the endonuclease domains acting as "scissors," cut out the region dictated by the gRNA. CRISPR is a flexible tool for genome manipulation because Cas enzymes bind target DNA independently of their ability to cleave it; thus, when the endonucleases are inactivated, Cas9 becomes nuclease-dead Cas9 (dCas9) and can be used instead to amplify the expression of a gene by recruiting the activation domains to promoter regions. This allows it to stimulate transcription, thus triggering hyperproduction of a gene's mRNA and protein.[1]

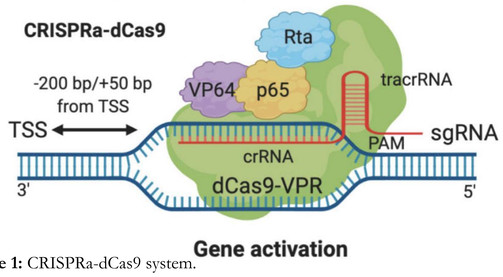


Figure 1: CRISPRa-dCas9 system.

This study targeted growth arrest-specific gene 1 (GAS1), a putative tumor suppressor gene that plays a role in growth suppression by blocking entry into the S phase and thus preventing the cycling of cells.[3]

This system has promising applications to several cancers. One of note is triple-negative breast cancer (TNBC), which makes up 10-15% of all breast cancers. Since TNBC lacks estrogen receptors (ER), progesterone receptors (PR), and HER2 protein, it is unresponsive to most hormonal and pharmacological therapies, making chemotherapy the primary choice available.[4] Recent studies have been exploring new forms of tailored therapies for TNBC, primarily focusing on the effects of inhibition through studying various agents such as targeted, antiangiogenic, and immune checkpoint inhibitors.[5]

Activation of this gene has not been well-explored; however, it has been shown that delivering a soluble form of GAS1 (tGAS1) decreases the number of viable MDA MB 231 human breast cancer cells,[6] indicating its ability to slow cell growth. Additionally, a genome-wide screen recently conducted by J. Antonio Gomez, David J. Segal, and Colleen Sweeney found that among the 18,885 promoters representing every known protein coding gene, hyperactivation of the GAS1 promoter most dramatically inhibited cell growth in the HCC1937 TNBC cell line.[2]

Two cell lines, HCC1937 and MCF7, were examined. HCC1937 cells are representative of TNBC because they are ER, PR, and HER2 negative.[7] In contrast, MCF7 cells are ER and PR positive and thus representative of hormone receptor-positive breast cancer, which makes up 80% of all breast cancers.[8] Studying these two cell lines yielded preliminary data on cell migration in cancer metastasis among a range of breast cancer subtypes.

Results

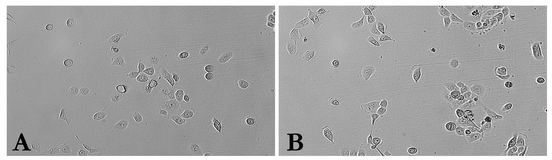


Figure 2: Untreated HCC1937 cells before (A) and after (B) 18-hour overnight incubation.

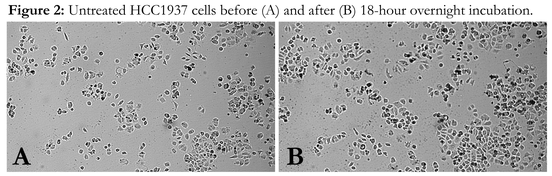


Figure 3: Untreated MCF7 cells before (A) and after (B) 24-hour overnight incubation.

Increased cell migration is characteristic of cancer cells and encourages metastasis. Figures 2 and 3 offer a preliminary examination of cell migration in various cancer subtypes.

View the live videos here!

Additionally, videos of each cell line over 18- and 24-hour periods give insight into cell motility. Note the rapid movement of the HCC1937 cells at 10X zoom and the MCF7 cells at 4X zoom.

Figure 4: Growth rate of untreated HCC1937 cells. Calculated over an 18-hour period by measuring the confluence every 20 minutes on three different points throughout the cell flask.

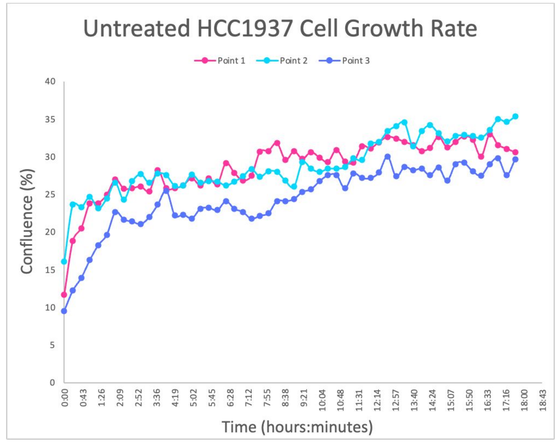


Figure 5: Untreated HCC1937 cells. Images taken over an 18-hour period.

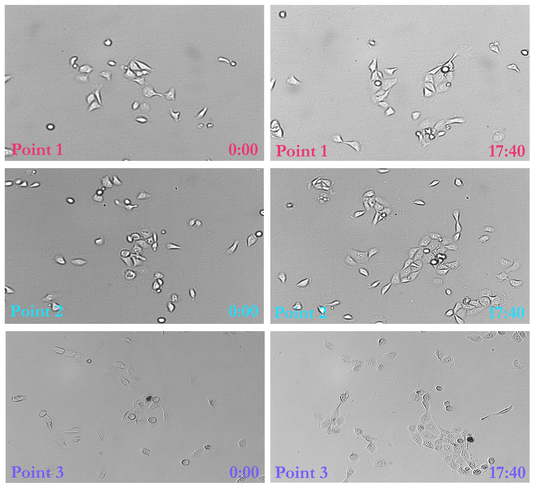


Figure 6: The average growth rates of untreated MCF7 and HCC1937 cells. Calculated by averaging the confluence across the three points (shown in figures 5 and 7) throughout the cell flask every 20 minutes.

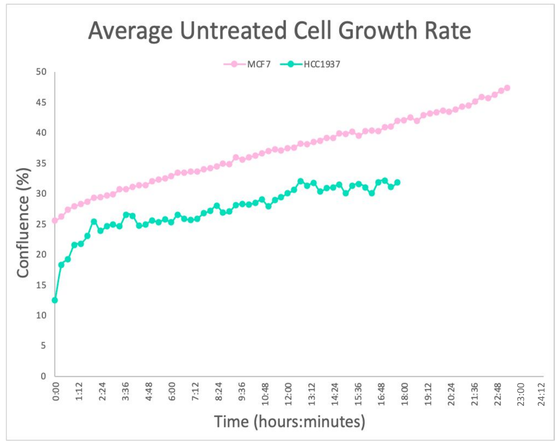


Figure 7: Untreated MCF7 cells. Images taken over a 24-hour period.

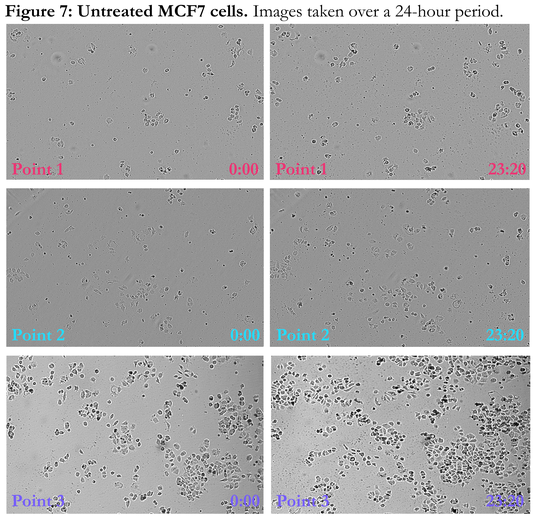
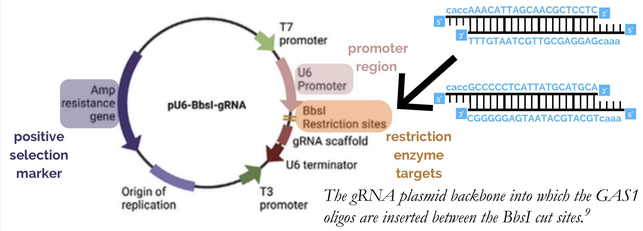
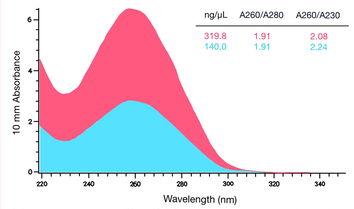


Figure 8: Designing and engineering a system to characterize the amplification of dormant tumor suppressor genes. After an intensive process that required many protocol adjustments, this novel system has been optimized to yield a high concentration of gRNA plasmid DNA that, once transformed with competent *E. coli*, yields colonies with the custom-engineered gRNA plasmid containing the promoter-targeting sequence.



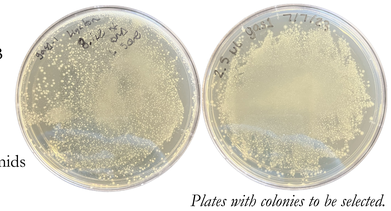
The gRNA plasmid backbone into which the GAS1 oligos are inserted between the BbsI cut sites.[9]



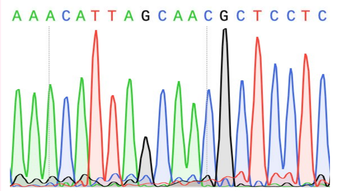
Nanodrop results. The DNA with a concentration of 319.8 ng/µL was used for the transformation.

1) gRNA plasmid creation: Sequence oligonucleotides complementary to two regions of the GAS1 promoter and insert into Addgene's PB-gRNA-Puro plasmid by digesting with the BbsI restriction enzyme. Nanodrop to check DNA concentration and proceed with the highest-concentrated DNA.

2) Bacterial transformation: Introduce gRNA plasmid into competent *E. coli* cells. Plate on LB agar ampicillin plates and incubate overnight. The ampicillin will positively select for colonies containing the plasmid; however, some will contain undigested plasmids that do not contain the oligo.

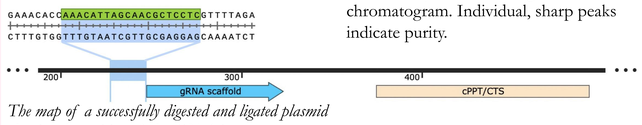


Plates with colonies to be selected.



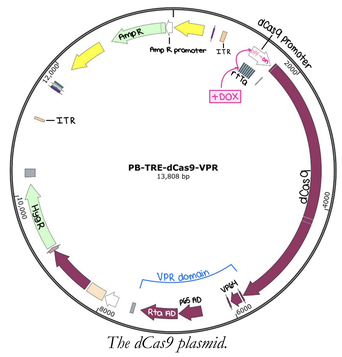
The DNA chromatogram results confirming the oligonucleotide sequence coding for the GAS1 promoter.

3) Sanger sequencing: Use Sanger sequencing to select for colonies containing plasmids that were correctly digested and ligated with the GAS1 oligonucleotide insert. If successful, the BbsI restriction sites will be absent and instead the GAS1 oligo will be inserted between the U6 promoter and the gRNA scaffold. Confirm the DNA's purity and the accuracy of the reading with a DNA chromatogram. Individual, sharp peaks indicate purity.

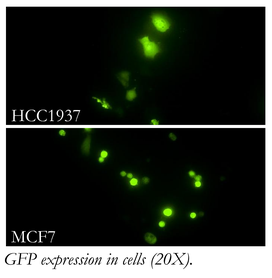


The map of a successfully digested and ligated plasmid showing the position of the gRNA insert.

4) dCas9 plasmid: Transfect the HCC1937 and MCF7 cell lines with the gRNA, dCas9, and piggyBac plasmids. The piggyBac plasmid uses transposase to paste the DNA of interest into the cells' chromosomes at any TTAA site. The dCas9 promoter is activated by doxycycline. Thus, the system is under control of a "switch" and will not amplify GAS1 production unless doxycycline is added.



The dCas9 plasmid.



GFP expression in cells (20X).

5) Controls: There are multiple controls to consider when treating the cells.
a) Leave some cells untreated.
b) Introduce the dCas9 and piggyBac plasmids to the cells without the gRNA plasmid to ensure that any growth changes are due solely to GAS1 amplification.
c) Use a GFP plasmid to measure transfection efficiency.

Conclusions

1. **Engineered a new model system to target dormant tumor suppressor genes using CRISPRa.**
   a. **This will be used to test whether activation of GAS1 (and other candidate genes) lowers cancer cell doubling rates.**
   b. **The system can be used to pair gene amplification with known chemotherapy agents to study whether their potency is increased.**
2. **Collected preliminary data to track cell migration and motility using a live-cell imaging microscopy system.**
   a. **Characterizing these behaviors in different cancer cell lines may aid in discovering positive associations between cancer invasiveness and cell motility, thus informing future investigations into the mechanisms aiding metastasis.**

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