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DOX Inducible IDH2 R140Q Expression in Stem Cells Results in Cell Death, Opposite of Cancerous Overgrowth

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INTRODUCTION

Mutations in the isocitrate dehydrogenase (IDH) 1 or 2 gene are found in approximately 23% of acute myeloid leukemia (AML) patients. IDH mutations have a neomorphic function and gain the ability to produce the novel molecule, D-2-hydroxyglutarate (D2HG).

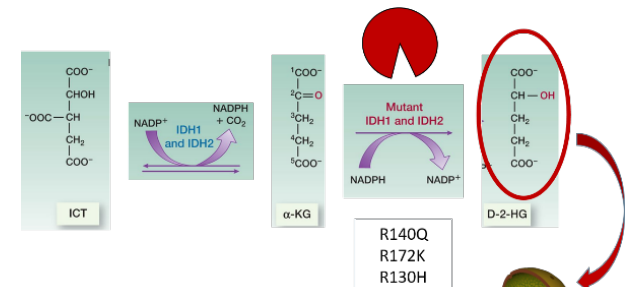


Figure 1

D2HG production has been shown to cause DNA and histone hypermethylation, which also occurs in leukemia, although the exact mechanism by which it does so is unknown. D2HG's ability to alter the epigenetic profile of DNA also affects the transcriptional profile. Histone methylation, for example, allows for tighter packaging of genetic material which prevents access by the transcriptome and thus prevents expression.

Our lab seeks to explain the link between D2HG, hypermethylation, and the phenotype of leukemia because we hypothesize that AML is caused in part as a result of altering the epigenome in a particular way. To explore this, our lab has developed a H9 stem cell line which has stably incorporated the specific IDH2 R140Q mutation behind a promoter that allows transcription in the presence of doxycycline (DOX) and before a green fluorescent protein (GFP) gene.

Our aim is to use this cell line to understand what D2HG specifically contributes that can lead to development of leukemia. General features of leukemia include increased cell proliferation and a block to differentiation. Therefore our lab uses these phenotypes as parameters to study the onset of cancer in the stem cells.

The purpose of this experiment was to characterize the pattern of growth in cells treated with DOX and to confirm that this was correlated with the production of the mutant enzyme. Our hypothesis was that GFP, as an indicator of IDH2 R140Q, would increase over time of treatment and that the number of cells would also increase, as is seen in leukemia cells.

METHODS



RESULTS

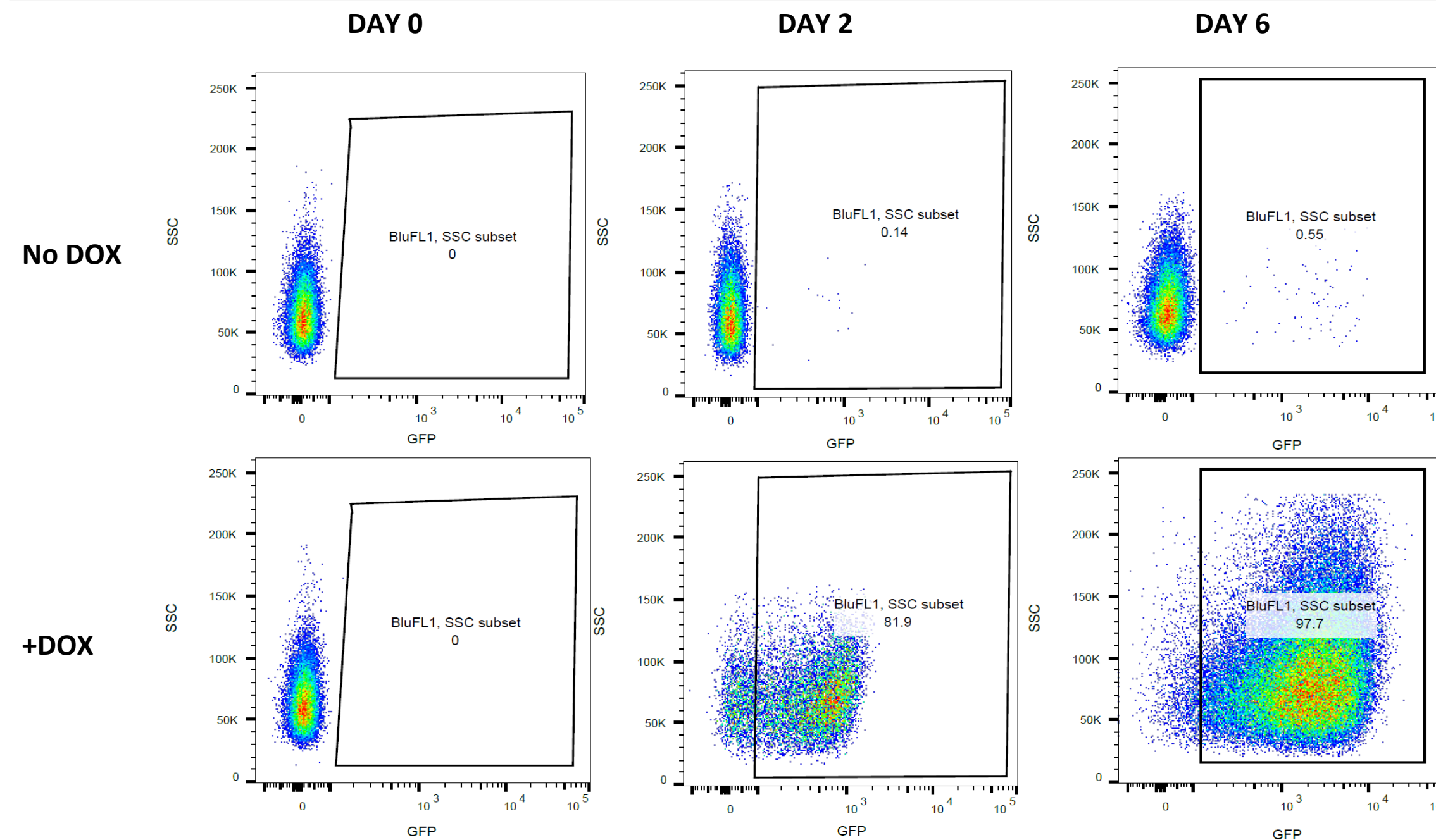


Figure 2: Cells were harvested at each timepoint and suspended in 1mL of PBS. The cells were spun down and washed in FACS buffer over three rounds and then filtered to obtain a single cell suspension. Flow cytometers were used to analyze the GFP positivity in the BLUFL1 range. Cells displayed were gated using SSC and FSC to determine live cells.

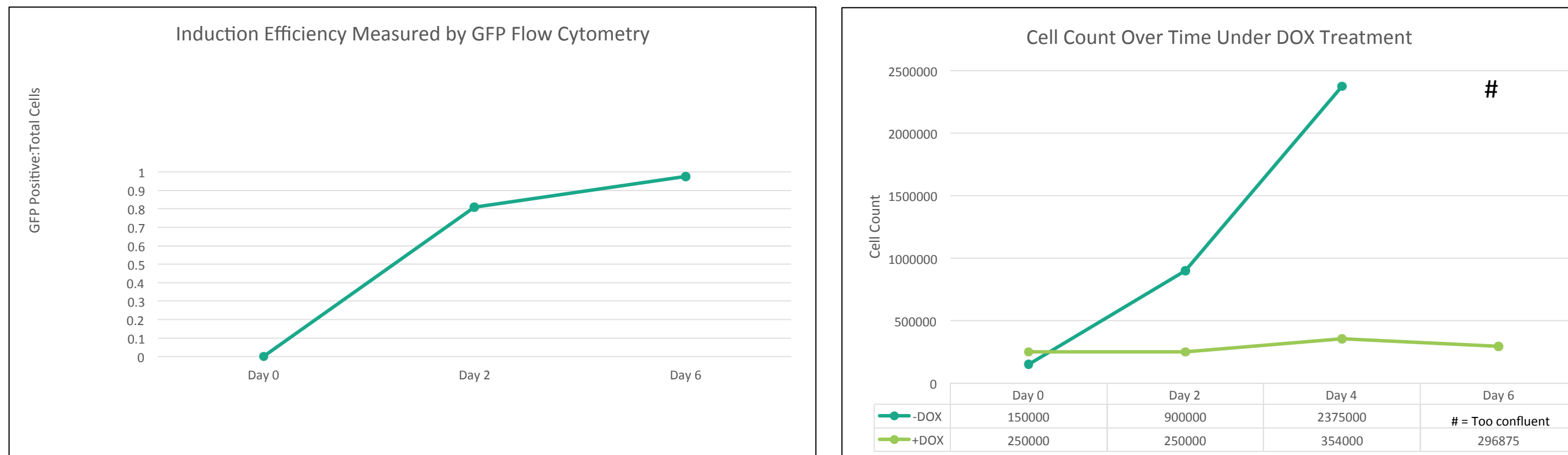


Figure 3: GFP positivity was determined by setting a boundary, as displayed in Figure 2, for -DOX cells which would be GFP negative and applying that boundary to the +DOX cells. The resulting fraction of cells that fell beyond that boundary were classified as GFP positive.

Figure 4: Cells were treated daily with a 1:1000 dilution of DOX from a 1mg/mL stock solution. At each timepoint, cells were counted by hematocrit. At the Day 6 timepoint for -DOX cells, cells were so confluent that they began to die and slough off. Therefore cells were split instead of being counted.

CONCLUSION

- DOX treatment of the H9 IDH2 R140Q cells causes the transcription and translation of GFP, which serves as a marker for the production of the mutant IDH enzyme.
- GFP positivity, and mutant IDH expression, increase over the course of treatment with DOX, which allows for more conclusive research over time.
- DOX treatment over time results in a decreased proliferation from the control condition in H9 cells that is the opposite of what is expected in a cancer phenotype.

FUTURE DIRECTIONS

- Cell growth is largely dependent upon type of tissue. Therefore it is likely that the decreased proliferation is a stem cell specific response to D2HG. Our focus in the future and currently is upon identifying which genes are differentially regulated between stem cells and leukemia samples so that we can modify the stem cells to become a model that more accurately represents progenitors to leukemia.
- Cancer phenotype is identified by increased proliferation and inhibited differentiation. In this experiment, we were able to understand the effects of D2HG on stem cell proliferation; however, future studies will follow up on the differentiation ability in the same cell line.
- In order to best mimic the environment that leukemia cells exist in, we need to perform future experiments to quantify the amount of D2HG that is produced by DOX treated cells. Then we can alter the concentration of DOX so that cells can produce a concentration of D2HG that is similar to that in leukemia samples.
- General future direction once this model has been adjusted includes determining the histone and DNA methylation sequence so that key methylation sites can be mapped to certain phenotypes.

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