Investigating a New Oncogenic Pathway in Rhabdoid Cancers

(Student Name)

Introduction

Rhabdoid tumors (RT) consist of aggressive malignant populations of cells that vary in location and are lethal to pediatric patients typically under the age of 1 years old. Some rhabdoid tumors known as malignant rhabdoid tumors (MRT) are found in the kidneys while other forms such as atypical teratoid rhabdoid tumors (AT/RT) are found in brain tissue and account for 10%-20% of tumors in children under the age of three (1). Due to the complexity and variety of tumor formations, no uniform treatments are available for rhabdoid cancers and the survival rates for patients with these cancers are very low. Almost all rhabdoid tumors can be identified by a common mutation to the *SMARCB1* gene that encodes the SNF5 subunit of the SWI/SNF protein complex (2). SWI/SNF is a chromatin remodeling complex – a major protein complex that regulates gene expression by manipulating the accessibility of DNA in the genome. Mutations to *SMARCB1* in rhabdoid tumors result in absence of SNF5 in the cell, which is the main driving mutation in almost all rhabdoid tumors. Loss of SNF5 leads to the cancer phenotype, indicating that *SMARCB1* is a tumor suppressor gene and shows the importance of SNF5-containing SWI/SNF complexes to combat tumor formation (3). However, in terms of cancer therapy, reintroduction of the tumor suppressor gene is not a viable strategy and many studies have set out to discover the pathways that are maintaining the cancer in the absence of SNF5, so that new cancer targets can be discovered and potentially inhibited in these deadly cancers.

Recently, two new pathways have been discovered that may be functioning in rhabdoid cancers, both of which depend on known oncogenes (2). Oncogenes are mutated genes that can form and maintain tumors while tumor suppressors are a classification of genes that encode

proteins that regulate cell cycling to avoid tumor states. One of the oncogenic pathways that was discovered involves the transcription factor AP-1, which stands for activator protein-1. Transcription factors are a family of proteins that interact with DNA for the regulation of which genes become activated and how much the genes are activated. AP-1 consists of a complex between two known oncogenes called FOS and Jun, and when FOS-Jun dimerize together they form an active AP-1 transcription factor that can bind to DNA sequences ("binding sites") in the genome specific to AP-1. In the most recent study, the presence of AP-1 binding sites at genes involved in maintaining rhabdoid cancer processes suggests that AP-1 may be helping to maintain the tumor state (2). If this is true, it implicates AP-1 as a new cancer target in rhabdoid tumors. Therefore, in this URECA proposal, I want to investigate how this newly discovered oncogenic pathway is impacting rhabdoid biology by using rhabdoid cancer cell lines. I hypothesize that inhibiting the binding of AP-1 to regulatory genes in rhabdoid tumor cells will decrease the cells' ability to survive – and that the cancer cells will lose necessary functions such as the ability to divide. This is based on the fact that the genes AP-1 binds to are genes required for cancer cell function (2). The findings from this study will help in providing evidence that AP-1 is an important protein by which cells with deficient *SMARCB1* function. These first experiments may uncover future targeted pathways for therapies in pediatric rhabdoid tumors.

Background

This proposal aims to determine whether cell lines derived from patients with rhabdoid cancers require AP-1 as a factor that helps the cancer cells continue to be able to divide and grow. The strategy to assess this is to block the ability of AP-1 to bind to the genes it controls. Since AP-1 is a transcription factor, if it cannot bind to DNA at the genes it controls, then the expression of those genes will be impacted. Two cell lines known as A204 (derived from

rhabdoid sarcoma) and G401 (derived from kidney rhabdoid) have been previously engineered in Dr. April Weissmiller's laboratory for this study. These cells have been engineered so that inhibition of AP-1 can be achieved through a tet-ON system – a system that induces the production of a protein fragment known as A-FOS when a chemical called doxycycline is introduced (see Figure 2, *appendix*). A-FOS acts as a competitor against normal FOS-Jun protein binding that created active AP-1. Therefore, when A-FOS is expressed the protein fragment will remove the ability of AP-1 to bind DNA (4). Having inducible control over whether AP-1 is a normal and functional transcription factor, or a non-functional transcription factor allows us to study the precise effects of AP-1's influence on cell division and cell processes. Importantly, both engineered cell lines, A204 and G401, have been previously verified to produce A-FOS upon doxycycline addition at the time I joined the laboratory of Dr. Weissmiller.

In addition, I have been trained in Dr. Weissmiller's laboratory to maintain cell lines in a tissue culture room. This involves instruction on cell line maintenance, cell culturing, and cell counting. The training was administered to allow me to perform this project independently. I have also started learning other techniques that may become important to this project including the generation of protein lysates and Western Blotting.

Purpose

My experiment aims to study how the inhibition of AP-1 function as a transcription factor affects the ability of rhabdoid tumor cells to divide and proliferate, which is a key function of any cancer cell. If AP-1 is important for rhabdoid cancer cell proliferation, then I hypothesize the inhibition of AP-1 function should impact the ability of the rhabdoid cancer cells to divide. I am focused on cell proliferation that is quantified through the ability of a cell population to increase in number from the cells present initially. For this experiment, engineered rhabdoid cell lines will be exposed to two conditions and counted over a seven-day period. The two conditions will be

the presence of doxycycline (+DOX) to turn on the AP-1 inhibitor called A-FOS, and the absence of doxycycline (-DOX) which should continue to have normal AP-1 functions. Again, if AP-1 is vital for cell proliferation in the rhabdoid tumor cell lines, the hypothesized result would be a decrease in the number of cells in the cell population treated with doxycycline (see figure 3, appendix for hypothetical graph).

Methods

Both cell lines (tet-A-FOS-A204 and tet-A-FOS-G401) have been engineered in Dr. Weissmiller's laboratory. For experiments pertaining to the engineered G401 cells, I will set up two experimental plates containing 250,000 cells in DMEM base media supplemented with 10% tetracycline-approved fetal bovine serum and 1% Penicillin/Streptomycin. One sample will receive 1 µg/ml doxycycline to induce A-FOS and the other sample will receive no doxycycline to serve as the control. For experiments pertaining to the A204 cells, I will plate 250,000 cells in RPMI media supplemented as above for G401 cells. For these cells, one sample will receive 1 µg/ml doxycycline and the other will receive no doxycycline. All plates will be incubated at 37 °C with 5% carbon dioxide. Over the course of seven days, I will count the cells in each treatment to determine the total number of cells present (see figure 1, appendix for the experimental procedure). On day 2 and day 4, I will replenish the cells with fresh doxycycline (+DOX treatment) or maintenance media (-DOX treatment). Cells on the final day will be counted and harvested from plates. For counting the cells, phosphate-buffered saline is utilized to wash the cells on the plate, and cells are extracted with 0.25% trypsin, an enzyme that degrades proteins that adhere cells to surfaces. Cells are then harvested, counted on a Countess II cell counter, and all values recorded. For each of the two cell lines, this experiment will be repeated three independent times to assess the reproducibility and variation in results.

References

(1) K. F. Ginn, A. Gajjar, Atypical teratoid rhabdoid tumor: Current therapy and future directions. *Front Oncol*. **2 SEP** (2012), , doi:10.3389/fonc.2012.00114.

(2) C. A. Jones, W. P. Tansey, A. M. Weissmiller, Emerging Themes in Mechanisms of Tumorigenesis by SWI/SNF Subunit Mutation. *Epigenet Insights*. **15** (2022), , doi:10.1177/25168657221115656.

(3) J. A. Biegel, L. Tan, F. Zhang, L. Wainwright, P. Russo, L. B. Rorke, Alterations of the hSNF5/INI1 Gene in Central Nervous System Atypical Teratoid/Rhabdoid Tumors and Renal and Extrarenal Rhabdoid Tumors1. *Clinical Cancer Research*. **8**, 3461–3467 (2002).

(4) L. Casalino, F. Talotta, A. Cimmino, P. Verde, The Fra-1/AP-1 Oncoprotein: From the "Undruggable" Transcription Factor to Therapeutic Targeting. *Cancers (Basel)*. **14** (2022), , doi:10.3390/cancers14061480.

Appendix

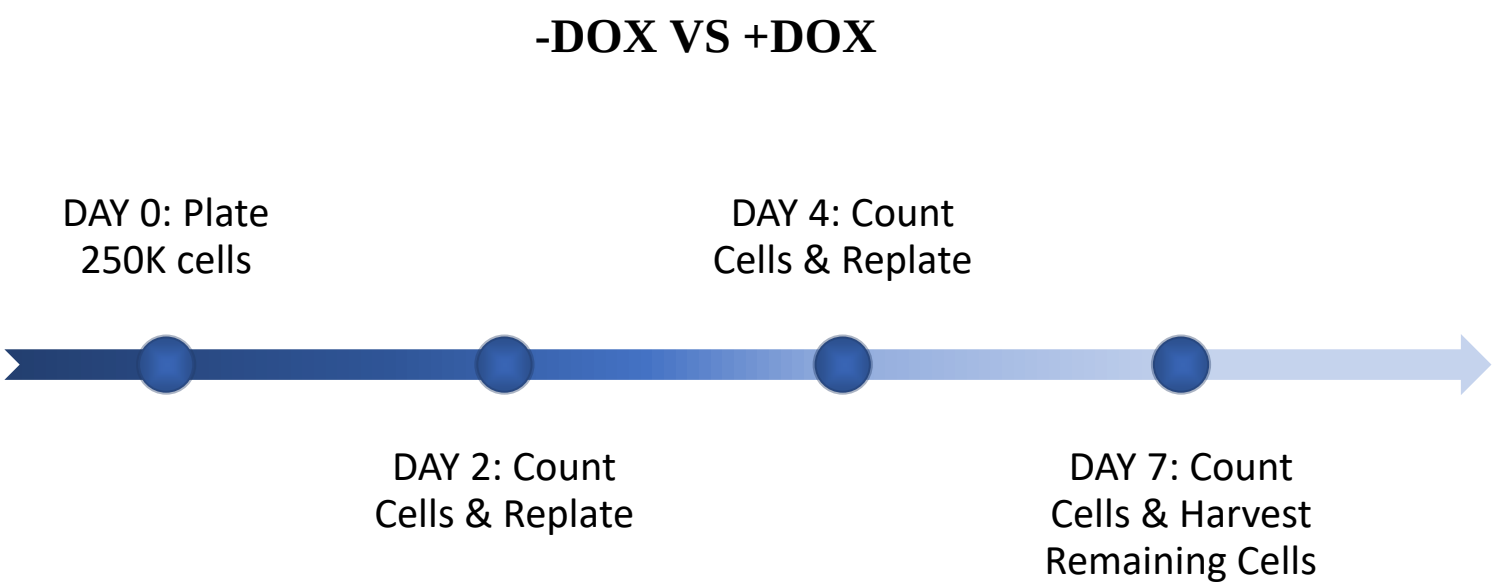
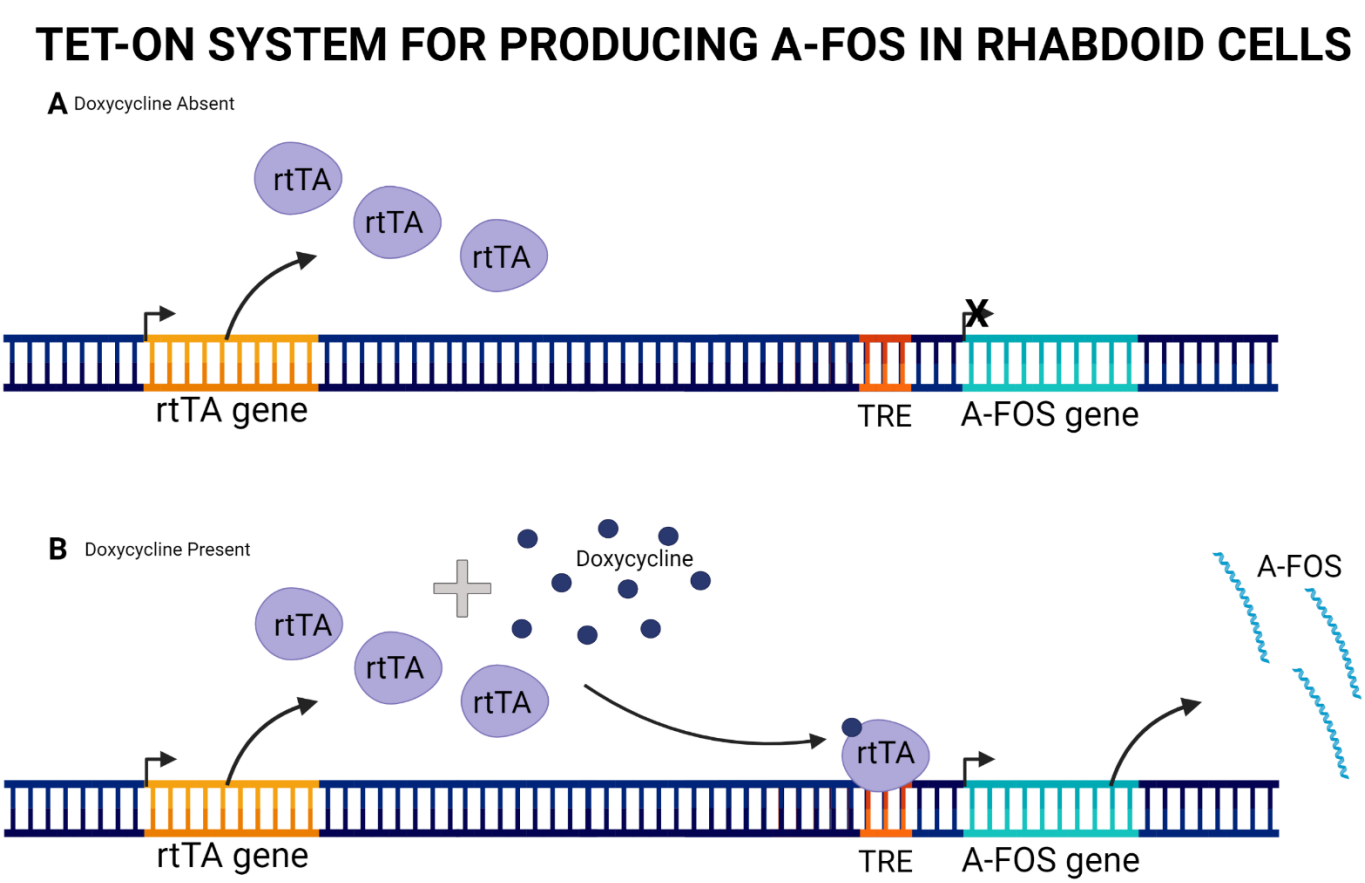


Figure 1. Proposed Experimental Design Process. This process will be reproduced a minimum of three independent times for my experiment meaning this experiment will contain n=3 biological replicates. The absence of doxycycline (-DOX) acts as the control for AP-1 to function normally in the cells while the presence of doxycycline (+DOX) is the treatment utilized to block the functionality of AP-1. Fresh media and doxycycline are supplied to cells on days 0, 2, and 4. Both A204 and G401 cells will be placed in both conditions, -DOX and +DOX, and then counted for total cell numbers.



Created with BioRender.com

Figure 2. Inducible protein system that has been engineered into two rhabdoid cell lines. . G401 and A204 rhabdoid cell lines have been engineered previously so that they contain the full DNA sequences shown in this image, which is a typical Tet-ON genetic engineering approach. The Tet-ON system allows for controlled production of the protein fragment A-FOS by adding doxycycline to the engineered rhabdoid cell lines, A204 and G401. (A) The rtTA activator is expressed from its own gene, however, it cannot bind to its specific sequence called tetracycline response element (TRE) to promote gene expression. Therefore, the absence of doxycycline does not allow the rtTA protein to produce A-FOS. (B) The rtTA gene is producing rtTA proteins that can interact with doxycycline to regulate the gene responsible for making A-FOS. Once rtTA

binds with doxycycline, rtTA can bind to the TRE, and expression of A-FOS is achieved. Therefore, in the presence of doxycycline, A-FOS can be produced to inhibit AP-1 function.

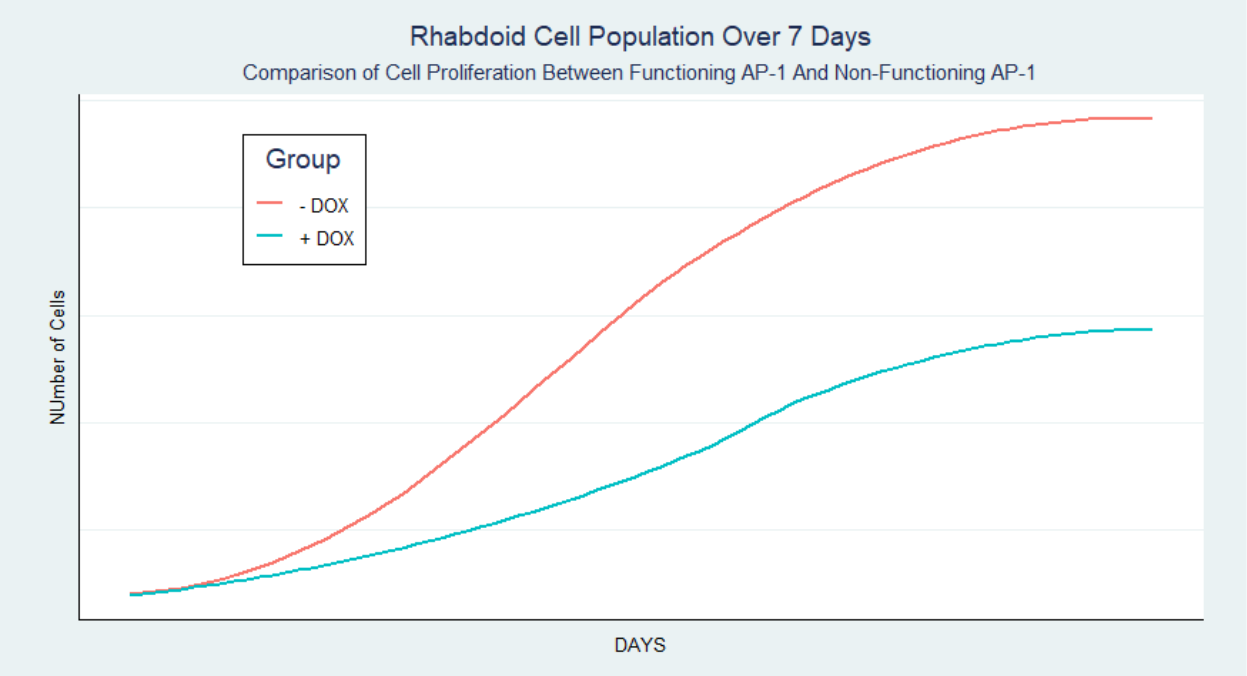


Figure 3. The proposed result if AP-1 is necessary for rhabdoid cell proliferation. This is only one possible outcome and represents how the graph will look if the cancer cells need AP-1 for proliferation. In this scenario, the +DOX treatment group has experienced a drop in cell numbers as they are exposed to doxycycline to induce the production of A-FOS which results in AP-1 losing the ability to bind to DNA. The -DOX treatment preserves AP-1 functionality and acts as the control curve for comparison.

Proposal Timeline

January 30th - February 10th: Maintaining and culturing the cell lines. Replicate 1, G401 cells: Perform treatment with doxycycline and record cell counts.

February 13th - February 24th: Analyze replicate 1 and determine if any changes to the experiment need to be made**.** Replicate 2-3, G401 cells: Perform two independent treatments with doxycycline and record cell count in the tissue culture lab.

February 27th - March 3rd: Graph all data for G401 cells, interpret results. Thaw out engineered A204 cells to have a second cell line for analysis.

March 6th - March 17th: Maintaining and culturing the cell lines. Replicate 1, A204 cells: Perform treatment with doxycycline and record cell counts.

March 20th - March 31st: Replicate 2-3, A204 cells: Perform two independent treatments with doxycycline and record cell count in the tissue culture lab.

April 3rd - April 14th: Analyze and interpret results, and repeat any necessary replicates.

April 17th - April 28th: Analyze new replicates and finalize data and graphs for presentation.

Timeline Table:

	January	February	March	April
Cell Culturing	**X**	**X**	**X**	
Data Analysis		**X**	**X**	**X**
Additional Replicates				**X**

Table 1. Proposed monthly activities as described in Proposal Timeline.

Collaboration with Faculty Mentor

Dr. April Weissmiller has and will continue to have a vital role in providing technical instructions, project guidance, mentorship, and data analysis for the time I am in her laboratory. With her extensive knowledge of tumorigenesis in pediatric cancers, she will be able to continue to be my mentor and supporter of my project as I perform my work in her lab. Regular weekly meetings will occur with Dr. Weissmiller allowing for the exchange of my progress in my project. This project directly benefits the research Dr. Weissmiller has performed concerning SWI/SNF mechanisms and rhabdoid tumors.

Budget Request and Justification

This project relies heavily on tissue culture work and cell maintenance/ cell number counting. We are requesting the following: Invitrogen Countess Cell Counting Chamber slides (quantity:2) $138.84 each, totaling $273.68 to cover the expense of the slides to be used for this project.

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