Investigating the Effect of AP-1 Inhibition on Cell Migration in Rhabdoid Tumor Cells

(Student Name)

Introduction

Malignant Rhabdoid Tumors (MRT) are aggressive pediatric cancers that typically affect infants, found in the kidneys, soft tissue, or liver (1). Almost all tumors of this cancer have a common mutation that results in the loss of the *SMARCB1* gene, which leads to the formation of these tumors, indicating the gene functions normally as a tumor suppressor (2). Gene reintroduction is unfeasible as a therapeutic pathway, so trying to figure out other pathways that maintain the cancer is worth exploring. One example and the focus of this project is based on evidence that the transcription factor AP-1 (activator protein-1) functions in an oncogenic pathway, maintaining the tumors, as evidenced in prior studies from the Weissmiller lab (1). Any "oncogenic pathway" means that the key proteins or events in the cell are pro-cancerous and help the cancer remain that way.

AP-1 is a dimeric protein, composed of two proteins from either the FOS or Jun family of proteins that come together to form AP-1 (2) and once AP-1 is formed it is a full transcription factor, which is a protein that binds to DNA across the genome at specific sites to help regulate expression of the genes that it binds to. In this case, the evidence suggests AP-1 binds to genes that are involved in cell migration (1), which is the ability of cells to move and have locomotion. This is a cancer hallmark, and cancer cells have an increased ability to migrate away from their location, which allows them to invade other tissues or cells in the body.

Background

This proposal aims to explore the role of AP-1 in MRT cell migratory ability using engineered cell lines where AP-1 binding to DNA can be inhibited. Two MRT cell lines, A204, and G401 were previously engineered in the Weissmiller lab to allow inducible expression of the A-FOS protein (Figure 1, *appendix)*, which inhibits AP-1 binding to DNA by competing with other FOS/Jun family members that normally dimerize to form AP-1 (Figure 2, *appendix*). A-FOS expression is induced using a Tet-ON system with the introduction of the chemical Doxycycline (Figure 3, *appendix*), and over the Summer of 2023, I learned how to work with the Tet-ON system by using this chemical induction method. Inducing genetic inhibition of A-FOS like this is important because we need to be able to only inhibit AP-1 for a specific period in which we can measure an effect, therefore we do not want to have A-FOS expressed in the cells all the time.

Purpose

The proposed project aims to study the effect of inhibiting AP-1 function on cell migration across two MRT cell lines. If AP-1 plays an important role in the migration ability of MRT cells as hypothesized, the inhibition of AP-1 will result in reduced motility of the cells as observed through a wound closure assay. The significance of the suspected reduction in cell migration following the inhibition of AP-1 will suggest that AP-1 is involved in an important cancer property that impacts cancer cell invasion and metastasis.

Methods

The two engineered MRT cell lines, Tet-A-FOS A204 and Tet-A-FOS G401, will undergo a wound-healing (cell migration) assay in which cells will be plated on a 12-well plate with their

respective maintenance media: DMEM base media for Tet-A-FOS G401 cells and an RPMI base media for Tet-A-FOS A204. This base media also includes 10% tetracycline-approved fetal bovine serum and 1% Penicillin/Streptomycin in each media type, a nutrient-rich artificial environment that supports the cells. Cells will be allowed to grow in a 37°C incubator with 5% carbon dioxide. The next day, once the cells have reached confluency (referring to the coverage of the plate with cells), the media of each cell line will be replaced with media supplemented with 20 μg/mL mitomycin C, an antibiotic that inhibits cell proliferation, or the division of cells, by inhibiting DNA synthesis. This enables the observation of cell movement without the confounding effect of cell proliferation. A scratch will be made vertically down the center of the plate using a pipette tip to represent a wound, removing the cells from the scratched area of the plate, wherein the migratory patterns of the remaining cells will be measured using an OLYMPUS IX83 Research Inverted Microscope. 10-20 mages of the simulated wound site will be taken upon the scratch being made and again 24 hours later to observe the migration. The percentage of wound coverage relative to the initial size of the scratch will be calculated for both cell lines and this will be used to analyze the effect that AP-1 inhibition has on cell mobility in MRT.

Collaboration with Faculty Mentor

I started working in Dr. Weissmiller's laboratory over the Summer of 2023 as part of the Undergraduate Experiential Training (U*NEXT) program. Since I will remain in the laboratory, we will continue to work together as we have during summer. In this lab, we have regular group meetings with other students who work on MRT in the lab, and at these meetings, I will present my progress and discuss how my experiments relate to the entire group. I also meet with Dr. Weissmiller to obtain advanced training on any techniques or discuss specific details of my

project. In these group and individual meetings, Dr. Weissmiller will assist me in learning to interpret my data. We also go over experimental design and how to quantify data to run statistics. These meetings are critical for my progress and will continue through the Fall semester.

Budget Justification

I am requesting $300 to offset the cost of purchasing tetracycline-approved FBS from Takara. This type of serum is specific for the Tet-on system, since normal FBS used in cell culture may contain tetracycline, a chemical that can function like doxycycline, leading to induction of A-FOS prior to experimentation. This FBS has been cleared of all tetracycline.

Fetal bovine serum for cell culture (tetracycline-free)

High-quality Tet System Approved FBS for cell culture has been **functionally tested*** to ensure that it permits the full range of tetracycline-regulated induction with well-characterized Tet cell lines. As the exclusive licensed distributor of tetracycline-controlled gene expression systems, only Takara Bio USA is certified to offer FBS that can ensure optimum induction with all tetracycline-inducible gene expression systems.

More

96 /100 949 Citations

References

(1) Jones CA, Tansey WP, Weissmiller AM. Emerging Themes in Mechanisms of Tumorigenesis by SWI/SNF Subunit Mutation. Epigenetic Insights. 2022; 15:25168657221115656.

(2) Wang X, Lee RS, Alver BH, Haswell JR, Wang S, Mieczkowski J, et al. SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation. Nat Genet. 2017;49(2):289-95.

(3) Eferl R, Wagner EF. AP-1: A double-edged sword in tumorigenesis. Nature Reviews Cancer. 2003;3(11):859-68

Timeline

September 4th – September 18th: Culture MRT cell lines and optimize conditions for the wound closure assay, learn how to use microscope.

September 18th – October 2nd: Perform first biological replicate of wound closure assay in both cell lines and learn how to analyze results.

October 2nd – October 9th: Perform second biological replicate in both cell lines, determine how reproducible the effect is compared to first replicate.

October 9th – October 23rd: Perform third biological replicate in both cell lines, analyze data to determine if AP-1 is involved in migration.

October 23rd – November 6th: Determine if earlier timepoints can be used for this assay (i.e. less than 24 hours) to see the timing of inhibition, if any. If positive, begin some experiments to test early effects as well.

November 6th – November 20th: Learn how to label and compile microscopy images using ImageJ software.

November 20th – December 4th: Make poster that can be displayed, write final report.

Appendix

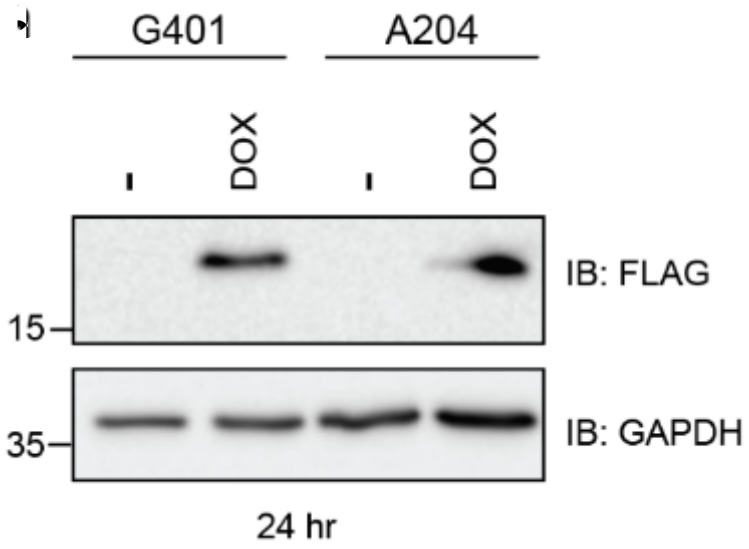

Figure 1. Western blot verifying A-FOS induction across the engineered cell lines. The results of this Western Blot verify the ability to induce the production of the A-FOS protein (FLAG-epitope tagged) across the engineered Tet-A-FOS G401 and Tet-A-FOS A204 cell lines in the presence of doxycycline for 24 hours. GAPDH is a housekeeping protein that is included in the blot as an internal validation across the cell lines with and without the presence of doxycycline, the expression of GAPDH should not change between conditions.

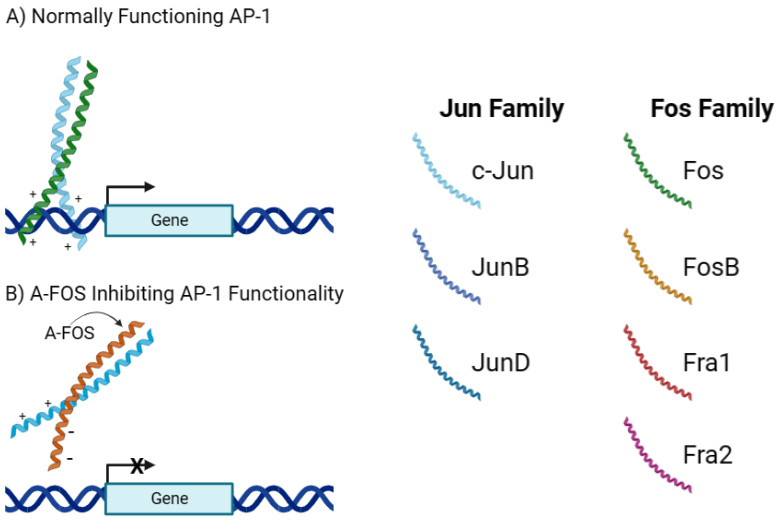

Figure 2. A-FOS-induced inhibition of AP-1. A) A-FOS competes with Jun family members as well as other FOS family members for incorporation into the dimer of AP-1. B) Due to the negative charge of the A-FOS protein, an AP-1 transcription factor with this A-FOS incorporated as a dimer is unable to bind to the DNA and initiate gene expression.

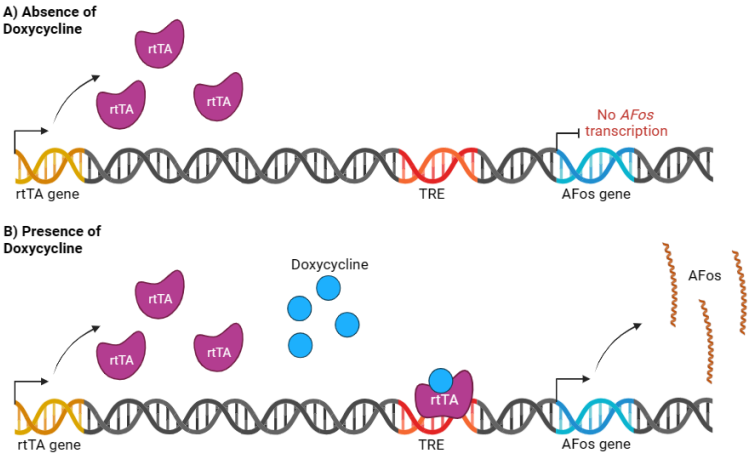

Figure 3. Tet-ON System for A-FOS Expression. A) In the absence of doxycycline, the A-FOS gene is not expressed because it is under the control of the TRE promoter element. B) When doxycycline is present A-FOS gene is induced because the doxycycline binds the activator (rtTA) that can then bind to the TRE element causing an increase in the expression of A-FOS.