Determining the Function of INI-1 in the Swi/Snf Complex- Contributing Research to Find a Cure for AT/RT Cancer

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Abstract

The Swi/Snf complex is also known as the chromatin-remodeling machine. The Swi/Snf complex plays a key role in the regulation of eukaryotic gene expression. The image below is a representation of the Swi/Snf complex. Each of the ten proteins plays an important part in the complex, due to their strong correlation to several cancer types. We currently are focusing on the INI-1 protein of the complex, which has shown to be correlated to the development of the atypical teratoid/ rhabdoid tumor (AT/RT). AT/RT is a highly malignant central nervous system tumor. Genetic studies have shown that deletion or mutation of the INI1 gene occurs in AT/RT lesions. Our lab is working to understand the biochemistry of the tumor suppressor INI-1 and its role within the Swi/Snf complex. If we are able to determine the function of INI-1 in the Swi/Snf complex, we will be better able to determine treatment for patients with this condition. The goal of my research is to determine which of the ten proteins that are part of the Swi/Snf complex directly interacts with INI-1.

Keywords: INI-1 SWI/Snf, AT/RT, Cancer Cure

Introduction

Our research is focusing on the ten proteins that are part of the SWI/SNF complex to determine which protein functions directly with INI-1. The SWI/SNF complex is also known as the chromatin-remodeling machine. The SWI/SNF complex plays a key role in the regulation of eukaryotic gene expression (Peterson & Tamkun, 1995). The image below is a punitive image of the SWI/SNF complex. Each of the ten proteins plays an important part in the complex, due to their strong correlation to several cancer types. We currently are focusing on the INI-1 protein of the complex, which has shown to be lacking in atypical teratoid/ rhabdoid tumors (AT/RT) (Tsai, et al., 2012). AT/RT is a highly malignant central nervous system tumor often misdiagnosed as some other type of pediatric embryonal tumor (Tsai, et al., 2012). Genetic studies have shown that deletion or mutation of the INI1 gene, which is located on 22q11.2, occurs in AT/RT lesions (Tsai, et al., 2012). In our lab, we are trying to understand the biochemistry of the tumor suppressor INI-1 and its role within the SWI/SNF complex (Ruhl, 2015). With regard to AR/RT, we are trying to determine the identifying characteristics of INI-1 in the complex, if some patients are missing a portion of or the entire amino acid sequence. If we are able to determine the function of INI-1 in the SWI/SNF complex, we will be able to determine how to treat patients with this condition.

Methods

We have determined that INI-1 is missing from the DNA structure in children who develop AR/RT at such a young age. Using polyacrylamide gel without SDS (native gel), we are investigating the shape and size of the protein. After determining the characteristics of INI-1, we will be able to move further into research of INI-1.

*=Mentor

We believe that this research will provide evidence that this type of juvenile cancer is present in patients who lack partial protein strands, as well as those lacking entire protein strands, allowing doctors to eventually treat patients with AR/RT more successfully. Based on the results of this study, our research could provide information that will allow pharmaceutical scientists to develop drug treatments specific to incomplete or missing protein strands. In turn, medical doctors will be able to prescribe a more specific treatment plan for AR/RT patients based on this information. Currently, doctors are using the same treatment for all patients with this condition. At this time, there is only a small chance of curing patients with AR/RT using available treatment options. Pharmaceutical scientists and medical doctors need additional information about the missing or partially missing protein strands known to be associated with this type of cancer in order to develop successful treatment therapies for patients. Our research will be able to provide the medical community with a better understanding of the cause and treatment of this condition.

The fundamental part of the research involves the proteins in the SWI/SNF complex that associate with INI-1. Each protein in the SWI/SNF complex contains information about INI-1 that we can use to determine the function of INI-1.

To begin our research, we will use protein expression to selectively grow a bacterium known as BL21. We will transform expression plasmids into BL21 by first incubating the bacterium overnight (Biolabs, 2015). The next step in our research is purification of the protein. We will be using Histidine affinity purification to purify the protein. Histidine affinity purification uses 6 histidines residues to produce recombinant proteins (Douglas Hayworth, 2015). Nickel, copper, and cobalt are utilized in the histidine purification process. Histadine purification provides a way to specifically purify or detect recombinant proteins without using probes or a protein-specific antibody (Douglas Hayworth, 2015). The third step in our research is to individually incubate each protein with INI-1. The incubation process will allow us to determine which protein is directly associating with INI-1 in the SWI/SNF complex. If the protein does not attach to INI-1 during the incubation process, we are able to conclude that the specific protein does not interact directly with INI-1 in the complex. By determining which protein is interacting with INI-1 directly, we will gain a better understanding of the role in INI-1 in the complex, and furthermore, its function in AT/RT.

After the incubation process, we will begin sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE is a technique for separating proteins based on their ability to move within an electrical current, which is a function of the length of their polypeptide chains or of their molecular weight (A Thermo Fisher Scientific Brand, 1989). This is achieved by adding SDS detergent to remove secondary and tertiary protein structures and to maintain the proteins as polypeptide chains (A Thermo Fisher Scientific Brand, 1989). Through amino precipitation, SDS-PAGE, we will run the combined proteins through the gel to attempt to separate the INI-1 from the other combined protein in the SWI/SNF complex. If INI-1 stays in contact with a certain protein, we will be able to determine that INI-1 associates directly with that specific protein in the SWI/SNF complex. We will

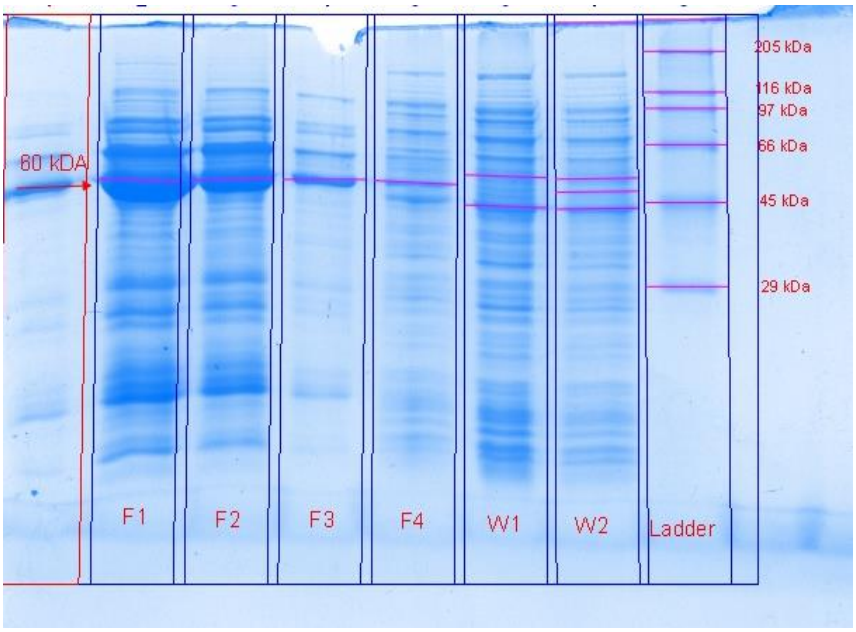

Figure 1 - The protein gel represents that BAF-60 protein was expressed

repeat this process nine times; each time we will combine a different protein from the complex with INI-1.

Progress to Date

I have been able to successfully run an SDS Page gel for BAF-66 protein and have started on BAF-155 protein. BAF-66 protein was successful and I will continue my research in the fall by expressing different proteins.

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