DNA Binding Domain of INI-1 1-141 in Atypical Teratoid Rhabdoid Tumors

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Abstract: Atypical teratoid rhabdoid tumors (AT/RT) are a devastating form of pediatric brain cancer. Due to the lack of current treatment for this cancer, the percent survival rate amongst children who are diagnosed with this cancer is only 10%. The expression of AT/RT cancer has been linked to the absence of a protein within the SWI/SNF complex called INI-1. Our lab has shown that INI-1 binds to DNA in the N-terminal half within amino acids 1-186. Because DNA binding domains typically do not require 186 amino acids, we hypothesized that there is a smaller binding domain within the 1-186 binding region. This paper shows the expression and purification of several N-terminal truncations of INI-1.

Keywords: Biochemistry, Protein Expression, DNA Binding, AT/RT, Cancer

Introduction

Atypical teratoid rhabdoid tumors (AT/RT) are fatal forms of pediatric brain cancer. AT/RT is expressed in the cerebellum of the brain, often leading to loss of basic motor skills (Chen et al.). Current treatment of AT/RT tumors are ineffective, leaving patients with very little chance of surviving for more than a year after diagnosis (Chen et al.). Children under the age of three who are diagnosed with AT/RT have a 10% survival rate (Fischer-Valuck et al. 2016).

Expression of AT/RT has been linked to the absence of a protein called INI-1 within the SWI/SNF complex (Hah et al. 2010). The SWI/SNF complex is responsible for moving histones responsible for organizing DNA in the cell's nucleus (Dallas et al. 2000). Various proteins are found within the SWI/SNF complex. Each protein within the SWI/SNF complex is correlated to a specific kind of cancer. For instance, if one protein is taken out of the SWI/SNF complex, breast cancer will occur. Likewise, if another protein, named INI-1, is absent from the SWI/SNF complex, AT/RT occurs. Therefore, proteins within the SWI/SNF complex are known to be tumor suppressing proteins because they inhibit the formation of cancer tissues in the brain (Dallas et al. 2000). Since the absence of INI-1 protein is positively linked to the expression of AT/RT tumors, we focused our investigation on the INI-1 protein (Hah et al. 2010).

INI-1 protein spans 385 amino acids in length. Our lab has found that full-length INI-1 binds with DNA in region 1-186 of the protein, which is referred to as the N-terminal. These studies have also found that DNA does not bind to INI-1 in region 187-385, which is referred to as the C-terminal.

We hypothesized that there is a smaller binding domain within the 1-186 binding region. We expressed and purified four different binding domains: 1-141, 31-141, 31-186, and 1-125 to determine the minimal binding domain of INI-1.

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Table 1 – Running gel preparation

Substance	Amount for 12% gel prep (INI-1 full length)	Amount for 12% gel prep (INI-1 full length)
30% Acrylamide	9.00 ml	11.0 ml
1.5 Tris pH 8.8	5.60 ml	5.60 ml
Deionized H20	7.60 ml	5.60 ml
10% SDS	225 µL	225 ml
TEMED	11.25 µL	11.25 ml
APS*	112.5 µL	112.5 ml

* APS is made by dissolving 0.1 grams of Ammonium Persulfate into 1 ml of deionized water

Methods

Transformation of INI-1 Clones

To determine the minimal binding domain within the known DNA binding domain 1-186, we expressed and purified the samples: 1-141, 31-141, 31-186, and 1-125 that were already cloned. First, the INI-1 truncation samples were put on ice, and the BL21 *Escherichia coli* cells were set out to thaw. 20 µL of the BL21 E. coli cells were mixed 2 µL of the truncation samples in a tube. The new solution was placed on ice for 30 minutes and then placed into a 42 °C water bath for 30 seconds. Then, the tubes were put on ice for another minute and thirty seconds. Next, 180 µL of SOC broth was added to the tubes and put on a 37 °C water bath for one hour. The solution was then added to a 5 ml of Luria broth media with Kanamycin (10mg/ml) and grown up in a 37 °C shaker set at 250 RPM overnight.

Induction of Protein

3 ml of blank media and 2 ml of the protein solution from the overnight process was placed into a cuvette and read in a Beckman Coulter DU 730 Spectrometer. The starting density OD reading was 0.06. Then, the 5 ml of blank media and protein were poured into four separate 400 ml flasks of Luria broth media. The solution in the flask was in the shaker until the OD reading reached 0.6. Then, 400 µL of IPTG was put into each flask to induce the sample proteins. After the IPTG was added, the cultures were incubated for another 2 hours in a 37 °C shaker set at 250 RPM. The samples were then transferred into centrifuge bottles and spun down for five minutes on a 5000 RPM (4068 RCF) Sorvall RC-5B Refrigerated Super-Speed Centrifuge with a GSA rotor. Excess media was poured off after centrifuging the samples, and the pellets of protein were stored at a temperature of -80 °C.

Sonication of the Cells

15ml of buffer with a pH of 7.5 was added to the samples of protein pellets. The new solution was vortexed until the pellets

Table 2 - Stacking gel preparation

Substance	Amount
30% Acrylamide	1.89 ml
1.5 Tris pH 8.8	2.49 ml
Deionized H20	10.2 ml
10 % SDS	150 µL
TEMED	15 µL
APS	150 µL

were completely dissolved in the buffer solution. Each beaker containing the new sample solution was put on ice. The contents of each beaker was sonicated on a Fisher Scientific Sonic Dismembrator FB-120 on a 10 second on and 10 second off time increment for 2 minutes. This process was repeated 3 times to ensure the cell membranes were completely opened, allowing the proteins to escape into the surrounding solution. After the sonication was completed, the samples were centrifuged for 15 minutes at 4 °C 15,000 RPM (32,571 RCF) in a Sorvall RC-5B Refrigerated Super-Speed Centrifuge with a GSA rotor. The samples were then placed into a -80 °C freezer.

Ni-Resin Affinity Chromatography

The resin was prepared in a 2 ml microfuge tube by adding 200 µL of Ni-NTA resin and 1ml of equilibration buffer pH 7.5 (10 mM Tris pH 7.5, 0.5 M NaCl, 0.5 mM EDTA, 10% glyceral, 0.1% NP 40). The contents of the tube were centrifuged in a VWR Micro 1814 centrifuge for five minutes at 4,000 RPM (1,300 RCF) and 2.8 °C. After centrifuging the sample, the supernatant was poured. Then, 1 ml of the equilibration buffer was added to the resin. This process was repeated three times.

To mix the protein sample with the Ni-Resin, the protein sample was pipetted into the resin and then the resin/protein sample was pipetted back into a conical tube. Preparing the column required inserting a small amount of glass wool into the a clean Kontes Flex-Column. 50 ml wash buffer was washed through the column.

In a cold room, the resin mixture was poured into the column. To ensure this process was done correctly, the column was watched closely to ensure a layer of resin remained on top of the glass wool. Once the wash was completed, 10ml of high salt elution buffer pH 7.5 was pipetted into the column. Elution fractions were collected after every 10 drops were released from the column.

SDS Polyacrylamide Gel Electrophoresis

To determine the purity of our proteins, a 15% SDS Page gel was run (Table 1).

To prepare the samples, 8 µL of the pH 7.5 buffer, and 6 µL of 20% SDS were added to a tube containing 16 µL of sample. Then, the contents of the tube were put in boiling water for 5 minutes. Then, the tubes were centrifuged for 12 seconds in a VWR Micro 1814 centrifuge. The gel apparatus

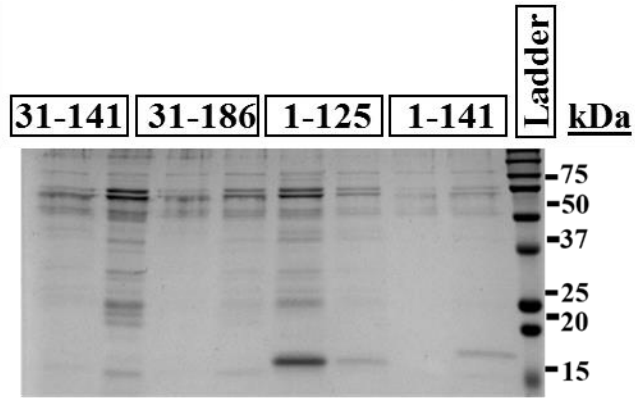

Figure 1 - The image above shows 2 elution fractions per INI-1 truncation. Truncation 1-125 has a thick band at 16 kDa, which demonstrates effective purification of the 1-125 truncation. The bands that appear in all of the truncation fractions are excess e.coli and unwanted proteins that were not eliminated in the purification.

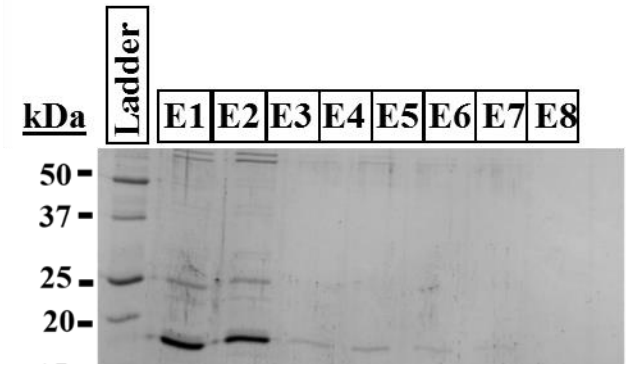

Figure 2 - The image above shows 8 elution fractions of the INI-1 truncation 1-141. The thick band at 18 kDa for elution 1 and 2 demonstrates a successful.

was set up and running buffer was poured throughout the gel running apparatus to ensure the gel ran properly. Using gel loading tips, 8 μL of the sample contents from each elution fraction were loaded into each of the wells. A protein ladder was inserted into one well of the gel because it contains known amounts of proteins and their molecular weights. The protein ladder serves as a reference point to ensure the gel ran properly. The lid of the gel running apparatus was placed back on and the machine was set at 130 Volts and run for 45 minutes. Once the elution fractions neared the bottom of the gel, the gel was rinsed with deionized water 3 times and then stained with a Thermo Scientific GelCode Blue Safe Protein Stain overnight. An image of the gel was taken the next day.

Results

We expressed and purified four truncation samples of INI-1. Figure 1 shows 2 elution fractions from 4 different INI-1 truncations. The expected molecular weights for INI-1 Truncations 31-141, 31-186, 1-125, 1-141 are 14 kDa, 19 kDa, 16 kDa, and 18 kDa respectively. 3 of the elution fractions expressed weakly, and 1 expressed well. The 15% gel did not yield a strong protein purification measurement for any of the INI-1 protein samples. We decided to re-run the next potential binding domain in the sequence, INI-1 1-141.

The INI-1 1-141 truncation was re-expressed. The thick band of protein on Figure 2 is at 18 kilodaltons. 18 kDa corresponds with the molecular weight of the truncation of INI-1 1-141. Therefore, the INI-1 1-141 truncation is a purified enough to perform DNA binding.

Discussion

If the DNA binding domain is found at fraction 1-141, this will give evidence that there are additional binding domains of smaller fractions within the larger binding domain 1-186. Finding a DNA binding domain within the 1-186 fraction will give researchers a better understanding of what proteins are involved in the expression of AT/RT tumors. Locating exact DNA binding domains of INI-1 will aid

researchers in developing a treatment for patients that are diagnosed with AT/RT tumors. Future studies can investigate the purification of INI-1 truncations 31-141, 1-125, and 31-186. Once these proteins are purified, the DNA binding protocol will be run to examine if these smaller fractions facilitate DNA binding.

Literature Cited

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