Purification of a Truncated Form of a Tumor Suppressor Protein INI1

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Abstract:

AT/RT tumors are a rare but fast developing tumor found in the central nervous system of infants. The cause of this tumor development is though to be correlated to the loss of a protein called INI1. The goal of this project was to purify the truncated C terminal end, containing amino acids 186-385, of the INI1 (186-385) protein. The methods used in the purification of this protein include: PCR and cloning of INI1 (186-385), culture, induction, sonication, and purification. A relatively pure protein was acquired but on order to get a completely purified sample, we will continue with sequential purification using other chromatography methods such as ion exchange chromatography. In the future we hope to get a pure enough protein to be able to get a crystal and analyze the structure. Hopefully this will unlock clues as the how the INI1 protein is lost and its role in the growth of the AT/RT tumors.

Introduction

Atypical Teratoid/Rhabdoid tumor (AT/RT) is a tumor found in the central nervous system in infants usually under the age of 3. It is very rare and grows rapidly once started in the brain or spinal cord (cancer.gov). About half of all AT/RT tumors are found in the cerebellum or brain stem. The cerebellum controls movement, equilibrium and posture in the body. While the brain stem controls other necessary functions such as breathing, heart rate and the nerves in sensory organs. It is thought that development of AT/RT is correlated to the loss of the protein SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1 (SMARCB1), a tumor suppressor gene more commonly referred to as INI1 (Childhood Central Nervous System 2014). INI1 is a part of the SWI/SNF protein complex that regulates transcriptional activity through chromatin remodeling. Changes in genes such as INI1 may lead to cancer due to the chromatin remodeling taking place (Birks 2008). These mutations are what cause the rapid growth of the AT/RT tumors and can either be spontaneous or be inherited from the infant's parents. They generally have no known cause in what makes them appear and cannot be prevented if the child has inherited it or if the gene has mutated. Previously AT/RT was thought to be a type of Medulloblastomas – another type of brain cancer – (Medulloblastomas 2012) but is now treated as a separate tumor with different treatment options (AT/RT - Atypical Teratoid Rhabdoid Tumor 2014).

Because this type of tumor is fast growing the symptoms develop quickly and worsen in a short amount of time. The symptoms include: headaches, vomiting, nausea, sleepiness, change in activity level, loss of coordination, and increase in the infants head size. Tests that can confirm the tumor growth are physical exam, neurological exam, MRI lumbar picture and INI1 gene testing (most commonly used in laboratories). Another common test is Immunohistochemistry (Childhood Central Nervous System 2014) in which antibodies are used the check for certain antigens in a sample and is used to tell if the tumor is

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actually an AT/RT brain tumor (Immunochemistry 2014). Recovery rates for infants over 3 are approximately 70 percent while infants younger than 3 have a 15 percent recovery rate. AT/RT is very difficult to cure and generally older children are able to handle the treatments better due to their bodies being more developed (AT/RT - Atypical Teratoid Rhabdoid Tumor 2014).

The basis of this project will be to purify at the truncated C terminal end, containing amino acids 186-385, of the INI1 protein.

Methods

1. PCR and cloning of INI1 (186-385) DNA: the INI1 protein was cut using the restriction enzymes, NdeI and BamHI and inserted into pET28a vector forming a new vector, pET28 INI1 (186-385). The new vector was then plated and the bacteria colonies grew. A restriction mapping gel of INI1 (186-385) was then run to see which colonies were successful in cloning. Those that were successful moved on to the culture stage of the project.
2. Culture and Induction: The colonies were then picked and grown in a LB buffer. Once grown the cells were induced to express INI1 (186-385) using isopropyl-beta-D- thiogalactopyranoside (IPTG). Samples were then spun down to collect the cells that had been induced.
3. Sonication: Cell membranes were broken open with high pitched sound frequencies to release proteins that were now set to express the INI1 (186-385). Once broken open the sample was spun down to get rid of the cell membranes and allow the purification process to be started.
4. Purification: In this experiment a polyhisitdine-tag purification method was used. The washes and elutions were run on a SDS-PAGE gel as shown if figure 2. The final lane of the gel shows how pure the protein is and what other proteins were purified along with it.

Results

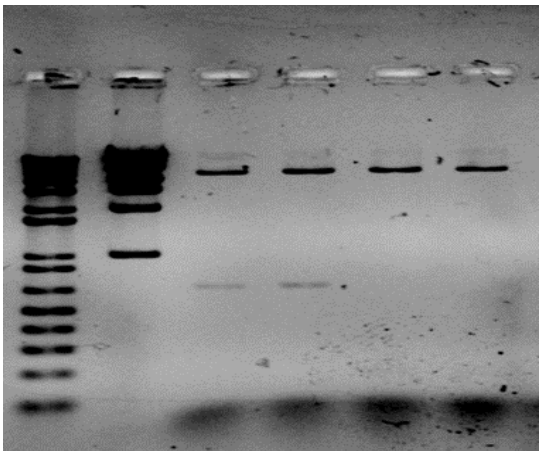

Figure 1 - This is the restriction mapping of the INI1 (186-385) vector. The vector was successfully cloned in lanes 3 and 4, as indicated by the dark bands that lie around 850 base pairs. With these successful clones, we were able to culture and induce them to move on with the purification process. (courtesy of biochemistry teaching lab)

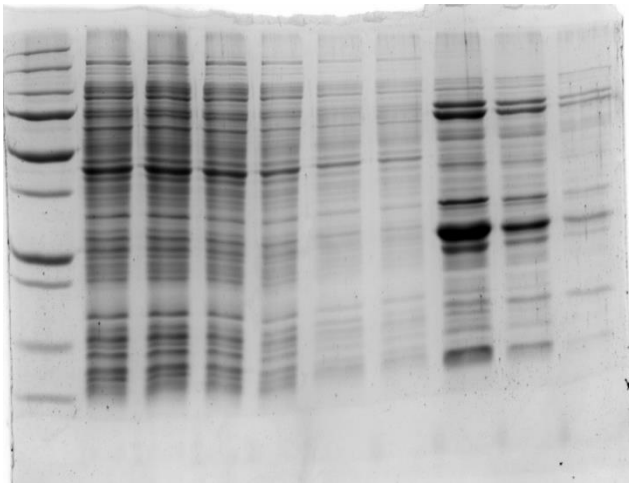

Figure 2 - This is the SDS-PAGE gel of the protein purification. Lane 1: kDa ladder;Lane 2: Pre-binding ;Lane 3: Flow through;Lane 4-6: Washes;Lanes 7-10: Elutions

The final lane of the gel shows the purified protein around 25,880 kDa however the actual protein is 23,880 kDa. This increase in kDa is due to the addition of the histidine tag that was used to bind the protein to the resin.

Discussion

Through this project we were able to make progress in the purification of this protein. However it is not totally purified due to the other bands present on the gel. To get a completely purified sample, we will continue with sequential purification using other chromatography methods such as ion exchange chromatography to get a more purified sample. This will cut down the number of extra bands on the gel and get closer to a basically pure protein. Once a purified sample is obtained, the sample will be used to form a crystal of the protein. Then by using x-ray crystallography we will be able to see what the structure of this half of the protein looks like. If this is achieved, the purified structure will be compared to other proteins to see what is similar, be able to learn more about how it forms the AT/RT tumors, and what may trigger it to develop in young infants.

Acknowledgements

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