Identifying Novel INI-1 Interacting Proteins

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Abstract: Atypical teratoid rhabdoid caner (AT/RT) is an aggressive embryonal cancer which affects the human brain's posterior fossa in early stages of life. The root cause of AT/RT originates during mRNA transcription due to a lack of INI-1 in the SWI/SNF complex. The research conducted was part of a base study analyzing the biological function of proteins which associate with the INI-1as to derive probable biological functions of the said protein. The result of two trials yielded an inconclusively diverse array of possible functions, more trials will have to be conducted to establish a solid precedence for future research.

Keywords: INI-1, Protein, Gene Ontology, mRNA Transcription, Cancer

Introduction

Atypical teratoid rhabdoid (AT/RT) tumors are an aggressive type of embryonal cancer which typically affects humans in the early stages of life. These tumors grow in the brain's posterior fossa and show a very high fatality rate after diagnosis. The cause of AT/RT originates in the SWI/SNF complex, which is responsible for removing and replacing histone proteins from DNA fibers during mRNA transcription.

The SWI/SNF complex consists of 9-12 individual proteins that are referred to as BRG1-associated factors, some of these factors include: BRG-1, BRM, BAF-250, BAF-170, BAF-180, BAF-155, BAF-60a, BAF-57, and BAF-47 (AKA INI-1) (Wang et al. 1996). Each protein serves a unique purpose and works together to remove and replace histones, yet some of some of these proteins are more impactful than others. Furthermore, the purpose and importance of some of these proteins remain undefined, several are directly linked to many types of cancers. For example, BRM is absent in ~20% of a broad range of human cancers. In contrast, BAF-180 is not correlated with any type of cancer (Glaros et al., 2007; Hah et al. 2010). Unlike BAF-180, INI-1 (aka BAF-47) is directly correlated with the presence of AT/RT brain tumors. Therefore, INI-1 is likely one of the most vital of the BRG1-associated factor as its absence or mutation can be fatal to young children soon after birth (Biegel et al. 1999).

In summary, this study aims to provide base knowledge of INI-1's specific biological function's through analyzing the function of proteins that associate closely with INI-1 using gene ontology analysis on a prepared INI-1 cell culture.

Methods

To grow INI-1, both an experimental and control variable were established. HeLa cells that had a flag tagged INI-1 comprised the experimental component and a wild type HeLa comprised the control. These cells were grown by removing/discarding the culture medium, rinsing the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution as to remove all traces of serum which contained trypsin inhibitor, then after adding the Trypsin-EDTA solution to the flask the cells were observed under an inverted microscope until cell layer was

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dispersed. Then, complete growth medium aspirate cells, and appropriate aliquots of the cell suspension were added to the new culture vessels. Finally, the cultures were incubated.

Once the cells had appropriately grown in the culture, they were stored in 1 mL conical containers until the lysing procedure. To lyse the cultures, the freeze-thaw method was used. The cultures were moved to a freezing cell suspension in a freezer and then thawed at 37°C. The cells repeatedly swelled and ultimately broke as ice crystals due to the imposed contractions. After lysing, the proteins were all then mixed with an INI-1 specific resin as to specifically select the INI-1. The resin was then rinsed to remove cellular waste. Once the HeLa wild type and INI-1 tagged cells were washed, half of the sample (enough for two trials) was mixed with loading buffer and boiled to uncoil/open the proteins.

As to ensure the cells were in a good state to be read by mass spectrometry, the samples were ran on a SDS page gel which pulls the proteins down by their individual weights. That half of the sample was sent to Dr. Steve Hartson (Oklahoma State University) where he ran mass spectrometry on the samples in two trials to determine small quantities of proteins that did not show up on the SDS page gel. Once the mass spectrometry was completed, the results were ran through T tests as to ensure significance across the trials. The significant results were then ran through a reputable gene ontology program (Gene Ontology Consortium) which yielded all significant biological functions associated with INI-1.

Results

The study revealed a number of biological functions that relate to INI-1: nucleosome disassembly, SRP-dependent cotranslational protein targeting to membrane, actin filament capping, ATP-dependent chromatin remodeling, viral transcription, nuclear-transcribed mRNA catabolic process [nonsense-mediated decay], translational initiation, rRNA processing, system development, and finally, actin cytoskeleton organization (figure 1). Of these results, everything except for nucleosome disassembly, nuclear-transcribed mRNA catabolic process [nonsense-mediated decay], and ATP-dependent chromatin remodeling were found to be novel functions.

Significant Biological Functions

- Nucleosome disassembly
- SRP-dependent cotranslational protein targeting to membrane
- Actin filament capping
- ATP-dependent chromatin remodeling
- Viral transcription
- Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay
- Translational initiation
- rRNA processing
- Actin cytoskeleton organization
- System development

Figure 1 - Results summation

Discussion

Upon reviewing the results, it is evident that many specific biological functions relating to transcription significantly appear several times in several different forms. These functions are: nucleosome disassembly, SRP-dependent cotranslational protein targeting to membrane, viral transcription, translational initiation, ATP-dependent chromatin remodeling, nuclear-transcribed mRNA catabolic process [nonsense-mediated decay], and rRNA processing. As these functions account for 70% of all the functions found, the accuracy of this study is reinforced as INI-1 is a known factor in the SWI/SNF complex and is thereby expected to have transcriptional properties. However, with this said, the list may or may not accurately represent INI-1 as it was acquired after running only two trials worth of HeLa cells. Further proofing of possible experimental inaccuracies arises from the remaining functions which were found, not relating to the expected transcriptional results. Some of these are: actin filament capping and actin cytoskeleton organization. As each of these functions are related to cell motility (Bear et al. 2002), they are unexpectedly variable from the likely transcriptional basis of INI-1. Therefore, this experiment can only be considered half completed, and the data shown, preliminary. The lab is equipped with enough HeLa cells to run another two trials which will likely reduce the experiment's results to a more accurate representation of INI-1's function. It is fair to speculate that after the completion of the second set of trials, more studies may be conducted with a basis of this study's findings.

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