Investigating the Alternative Splicing Patterns of RNA Binding Proteins (RBPs) in Breast Cancer

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Abstract

Ribonucleic acids (RNAs) in cells are associated with RNA-binding proteins (RBPs), and this project works with a specific type of RBPs, called heterogeneous nuclear ribonucleoproteins (hnRNPs), which contribute to multiple aspects of RNA metabolism, including alternative splicing, which is the process during gene expression, where a single gene is expressed as multiple isoforms that code for different proteins. This project investigates whether the genes that encode the hnRNPs are alternatively spliced, such that the regulators of splicing (i.e. hnRNPs) are themselves regulated at the splicing level. Alternative splicing of a given hnRNP would generate different versions of that hnRNP with various potential functions with regards to the alternative splicing of its targets, which could lead to diseases, such as cancer. This has not been extensively studied before, especially in the context of breast cancer. This project works with a panel of 12 hnRNPs, studying their alternative splicing patterns, primarily using RT-PCR and bioinformatics, and this is done on breast cancer cells as breast cancer is the most common cancer in Qatar. In addition to studying the alternative splicing patterns of these 12 hnRNPs, this project also narrows down the hnRNPs to one, which is hnRNPA2B1 based on many factors, and this hnRNP is further studied computationally. The ultimate aim of this research is to uncover breast cancer-specific alternative splicing of these hnRNPs, which could set the foundation for novel therapeutic approaches as the expression level of hnRNPs is altered in many types of cancer, suggesting their role in tumorigenesis.

Introduction

- Alternative splicing is the process in which one pre-mRNA (of a single gene) is processed into various isoforms that could code for multiple proteins
- We know that hnRNPs play an important role in alternative splicing, where they regulate the alternative splicing of hundreds of exons and introns in their downstream targets, but we hypothesize that their own pre-mRNAs are alternatively spliced.

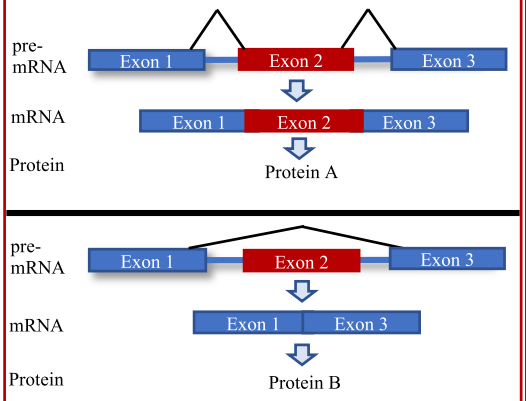


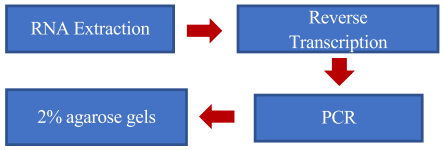
Figure 1: the potential alternative splicing events for hnRNPA2B1.

Proteins A and B are two different protein isoforms from the same gene (hnRNPA2B1), and may have different alternative splicing effects on their downstream effectors, which could lead to cancer. So, as the regulators of alternative splicing are potentially alternatively spliced, we propose that the regulators are regulated by the same process they regulate. However, the alternative splicing patterns of these regulators, the hnRNPs, has not been extensively studied, especially in the context of breast cancer, and therefore this project aims to investigate the alternative splicing of a panel of 12 different hnRNPs, and then narrow the list to one, on which further research is carried on, which is hnRNPA2B1. This research is done on breast cancer cells as breast cancer is the leading cause of cancer mortality in women worldwide, and it is by far the most common cancer in Qatar.

Materials and Methods

- Template DNA (breast cancer cell lines):
  - MDAMB231 (triple negative and claudin-low)
  - MDAMB468 (triple negative and basal)
  - T47D (ER+, PR+/-, HER2- and Luminal A)
  - MCF7 (ER+, PR+/-, HER2- and Luminal A)
- Primer Design

- RT-PCR



- Bioinformatics
  - POSTAR (correlations and anti-correlations)
  - XenaBrowser (phenotypic patterns and visual representations of the correlations)
  - UCSC Genome Browser (RBPs' binding to exon 2 of hnRNPA2B1)

Results and Discussion

1. Experimentally

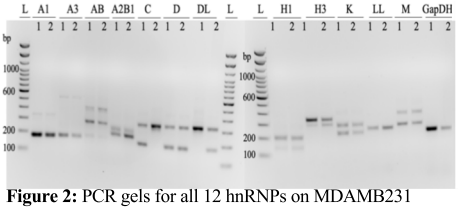


Figure 2: PCR gels for all 12 hnRNPs on MDAMB231 and T47D:
L = 100bp ladder, 1 = MDAMB231, and 2 = T47D

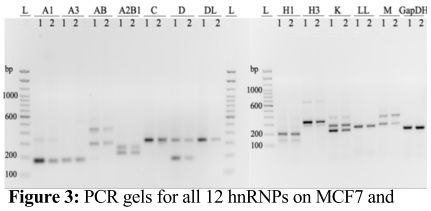


Figure 3: PCR gels for all 12 hnRNPs on MCF7 and MDAMB468:
L = 100bp ladder, 1 = MCF7, and 2 = MDAMB468

Table 1: Scoring system to narrow down hnRNPs

hnRNP	Alternative splicing?	Differentially spliced?	Differentially Expressed?	Functional Consequences Score	TOTAL: (max of 5)
A2B1	4.5	5	1	5	4.45
H3	4.5	5	2	4	4.25
M	4.5	2	1	5	3.55

Narrowed down to hnRNPA2B1

Figure 3: PCR Gel results for hnRNPA2B1 only

- Alternatively spliced at exon 2, therefore two isoforms: one including exon 2, and one skipping it. The one skipping it would lose the Nuclear Localization Signal (NLS), which is responsible for entry to the nucleus as it is encoded by a big part of exon 2.
- Functions: forms hnRNP particles with different hnRNPs, involved in the transport of specific mRNAs to the cytoplasm and miRNA sorting into exosomes, binds single-stranded telomeric DNA sequences and other RNA molecules, and plays a role in the activation of the innate immune response, and more.

2. Computationally

Kaplan Meier Plot, phenotypic patterns, the RBPs correlated and anti-correlated with the gene expression of hnRNPA2B1, and the RBPs binding exon 2 specifically.

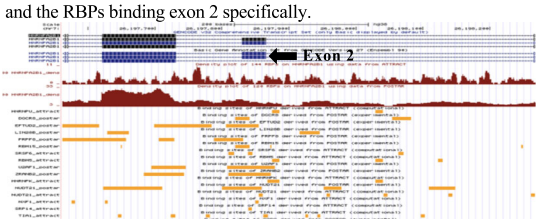


Figure 4: Data from the UCSC genome browser showing the RBPs that bind on and around exon 2 of hnRNPA2B1

Table 2: Top 5 RBPs from the UCSC genome browser

Rank	RBP
1	U2AF1
2	ZRANB2
3	EFTUD2
4	PRPF8
5	RBM15

Factors taken into account to rank RBPs: Function, origin of data, and binding on exon 2.

Conclusions

- 9/12 of the hnRNPs studied, at a 75% rate, were alternatively spliced
- The best hnRNP for follow up work was hNRNPA2B1, which is alternatively spliced at exon 2, encoding the NLS. There might be different functions this hnRNP encounters in the cytoplasm that makes it likely to be alternatively spliced to not go to the nucleus, and different patterns were studied. Finally, there was an extensive study on the RBPs binding the whole hnRNP, and exon 2 only, which led to narrowing down the RBPs.

Future Work

- Update the scoring system for the RBPs to include correlation between hnRNPA2B1's exon 2 presence and the expression of the RBP, and find RNASeq data for the top RBPs
- Carry out experiments to test the phenotypic effects, such as cloning and expressing, knocking down using siRNA, or overexpressing hnRNPA2B1
- Test the effects of the top RBPs found computationally. This can be done by overexpressing the top RBPs, and studying the splicing of hnRNPA2B1
- Study similar patterns for more hnRNPs.

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