Targeting RNA binding protein-dysregulation associated neoepitopes for triple negative breast cancers

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Abstract

Neoantigen-based cancer vaccines are largely individualized treatment modalities based on a patient's DNA mutations. However, tumor-specific neoantigen formation may also come about as the result of other downstream processes like splicing dysregulation and nonsense mediated RNA decay (NMD) that result in aberrant transcripts. Such processes can be regulated by RNA binding proteins (RBPs). We have shown previously that one such RBP, hnRNPM, preferentially binds to UG-rich motifs and suppresses cryptic splice sites. Knockdown of hnRNPM resulted in consistent upregulation of common aberrant cryptic transcripts across multiple cancer cell lines. With the aid of immunopeptidomics mass spectrometry, we identified a peptide produced by translation of an hnRNPM-associated cryptic transcript in triple negative breast cancer cell lines. This peptide is predicted to be a strong binder to human leukocyte antigen class I (HLA-I) and does not map to canonical protein sequences. This preliminary result will be followed up with further characterization of neoantigens produced by RBP dysregulations. The goal will be to develop a pool of clinically actionable neoantigens for individual patients by searching for RBP dysregulations that can predictably produce abnormal neoantigens.

Background

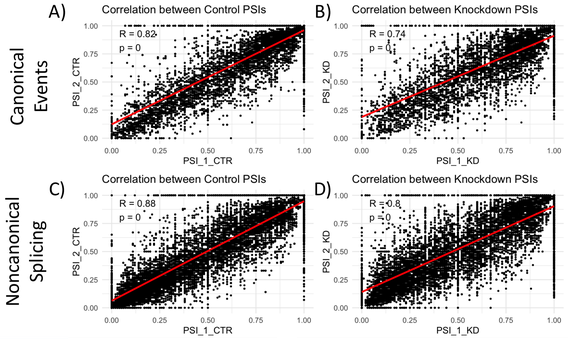
Our group has previously shown that knockdown of RBP hnRNPM in the HMLE mammary epithelial cell line results in aberrant transcript splicing. These noncanonical cryptic splice sites are thought to arise from expansion of intronic retrotransposon elements across the genome. hnRNPM binds and suppresses these pseudo splice sites, and in vitro-knockdown has been shown to result in the consistent inclusion of novel exon and retained intron events in target transcripts.

hnRNPM protects against the dsRNA-mediated interferon response by repressing LINE-associated cryptic splicing Zheng, Rong et al.
Molecular Cell, Volume 84, Issue 11, 2087 - 2103.e8

Effects of hnRNPM knockdown on splicing in TNBC cell lines

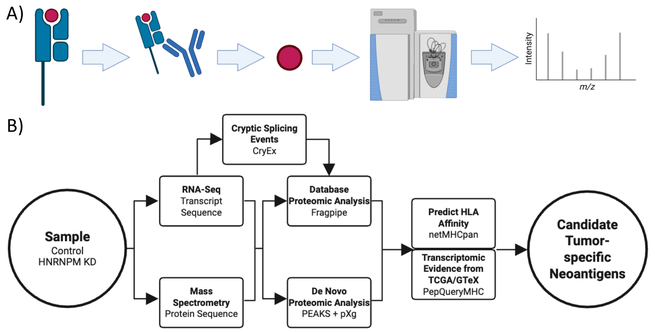
Experimental Design for downstream work with RNASeq and proteomics
Luciferase (control) or hnRNPM were knocked down in two TNBC cell lines: LM2, a lung metastatic cell line derived from MDA-MB-231, and SUM159.

Splicing quantification in LM2 control and LM2 hnRNPM KD



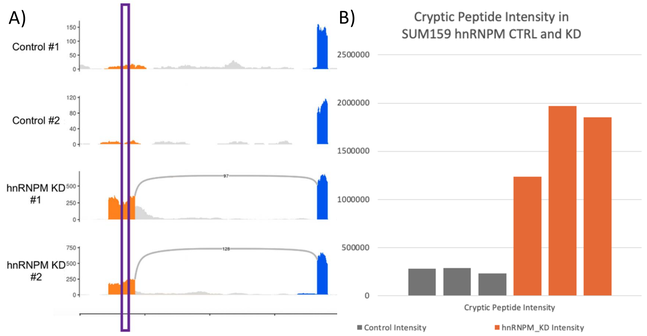
Splicing quantification consistent between duplicate experimental conditions
Quantification of splicing events uses a metric called PSI (Percent Spliced Index) that ranges from 0 to 1. These are correlations between two replicate RNASeq datasets from LM2. PSIs for splicing events that overlap with canonical exons are shown for controls **(A)** and hnRNPM knockdowns **(B)**, while PSIs for splicing events that do not overlap with canonical exons are shown for controls **(C)** and knockdowns **(D)**.

Proteogenomic workflow for identifying cryptic-splicing derived neoantigens



Proteogenomic workflow for identification of cryptic-splicing derived neoantigens from TNBC cell lines. A) Immunopeptidomics is used to profile peptides bound to the HLA-I antigen presenting complex **B)** RNASeq and immunopeptidomics data are utilized in a workflow that incorporates orthogonal methods of mass spectrometry analysis, followed up with tools for prediction of predicted peptide HLA affinity, and for tumor-specific peptide expression.

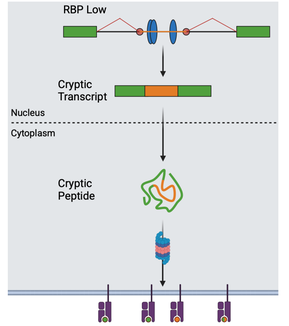
Results



Identification of a HLA-I presented peptide derived from non-canonical splicing and translation of a lncRNA A) hnRNPM knockdown results in the inclusion of an exonic region in the RNA transcript. Boxed in purple is the genomic region corresponding to the peptide **B)** Immunopeptidomics shows that the peptide's presentation on HLA-I is significantly upregulated by knockdown of hnRNPM in the SUM159 cell line.

Conclusions

- Workflow was able to identify cryptic splicing events, and subsequently identify a novel peptide that arose as a result of hnRNPM knockdown
- netMHCpan characterized peptide sequence as a strong binder to universally frequent HLA allele HLA-A*24:02, which is present on the SUM159 cell line
- However, analysis via PepQueryMHC suggested that this peptide is not expressed in a tumor-specific manner



Future Directions

- Modify and update workflow to identify additional HLA-presented cryptic splicing-derived peptides
- Test immunogenic capacity of candidate peptides against donor PBMCs
- Investigate other RNA Binding Proteins whose dysregulations may be able to result in similar abnormal peptide splicing and presentation

Acknowledgements and References

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References:

- hnRNPM protects against the dsRNA-mediated interferon response by repressing LINE-associated cryptic splicing. Zheng, R et al.
- pXg: Comprehensive Identification of Noncanonical MHC-I-Associated Peptides From De Novo Peptide Sequencing Using RNA-Seq Reads. Choi, S, Paek E.

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