The M1C/MYC Axis Reprograms Alternative Splicing Networks in Triple Negative Breast Cancer

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Introduction

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by limited treatment options, enrichment of cancer stem cells (CSCs), and poor outcomes, with only ~22% of patients with metastatic disease surviving 5 years and an average survival of ~3 years[1]. There is a critical need to define targetable pathways that sustain TNBC growth, stemness, and immune evasion.

Mucin 1 (MUC1) is aberrantly overexpressed in ~90% of TNBCs and comprises an extracellular N-terminal subunit (MUC1-N) and a C-terminal oncogenic subunit (M1C). The 72–amino acid M1C cytoplasmic domain contains a CQC motif that enables dimerization and interaction with MYC, linking M1C to epigenetic remodeling and CSC maintenance, while its role in RNA splicing remains unclear[2-5].

Our preliminary data indicate that M1C associates with nuclear RNA processing proteins and splicing factors and that silencing M1C alters spliceosome gene expression and splicing patterns in TNBC cells. These findings support a model in which an M1C/MYC axis acts as critical regulator of alternative splicing in TNBC. This study examines how targeting this axis and the splicing machinery may uncover novel therapeutic vulnerabilities in aggressive breast cancers.

Results

M1C associates with splicing proteins and regulates their expression in TNBC cells

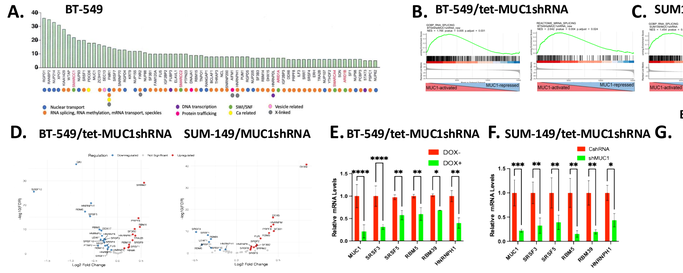


Figure 1. M1C associates with RNA-processing splicing factors – A. Rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) was performed using a M1C antibody on nuclear lysates from BT-549 cells, and mass spectrometry identified M1C interaction partners enriched for RNA-processing and splicing proteins. B. Analysis of RNA-seq data from BT-549 TNBC cells demonstrated that inducible M1C silencing is associated with regulation of the GOBP RNA SPLICING and REACTOME MRNA SPLICING gene signatures. C. For comparison, we analyzed SUM-149 TNBC cells and found that stable M1C silencing with a second MUC1shRNA#2 also associates with regulation of RNA splicing and spliceosome gene signatures .D. Volcano plots from RNA-seq data from tet-inducible M1C silencing in BT-549 cells and stable M1C silencing in SUM-149 cells further identified differentially regulated splicing factors. E,F. Silencing M1C in BT-549 and SUM-149 cells further identified suppression of genes encoding SRSF3,SRSF5,RBM5,RBM39 and HNRNPH1. G. Lysates from tet-inducible M1C silenced BT-549 cells were immunoblotted with antibodies against the indicated proteins.

Results

M1C regulates splicing factor expression by a MYC dependent mechanism

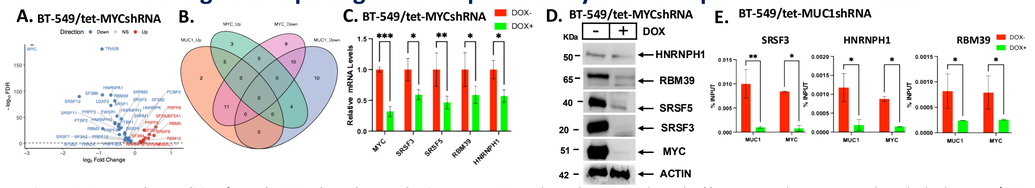


Figure 2. M1C regulates splicing factor by MYC dependent mechanism – A. M1C regulates the MYC pathway by (i) activating the MYC gene through the b-catenin/TCF4 transcriptional complex, and (ii) binding directly to the MYC HLH-LZ domain and thereby the MYC transactivation function[6]. RNA-seq analysis of MYC silenced BT-549 cells showed differential expression of multiple splicing factors. B. Comparison of RNA-seq data from MUC1 silenced and MYC silenced cells identified the suppression of ten common splicing factors. C, D. Immunoblot analysis of BT-549 cells expressing tet-inducible MYC shRNA, with or without doxycycline treatment for 7 days, showing reduced expression of the splicing factors SRSF3, SRSF5, RBM39, and HNRNPH1. Cell lysates were immunoblotted with antibodies against the indicated proteins. E. ChIP studies demonstrated occupancy of M1C and MYC at the promoter region of SRSF3, HNRNPH1 and RBM39 . Moreover, silencing M1C significantly decreased MYC occupancy at the promoter region of these genes.

M1C regulates Alternative Splicing in TNBC cells

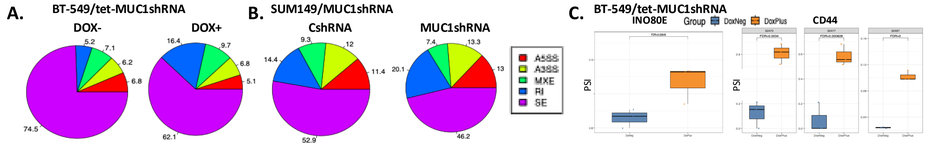


*Figure 3. M1C regulates Alternative Splicing in TNBC cells – A,B. rMATS analysis of RNA-seq data from BT-549/tet-MUC1shRNA cells cultured in the absence or presence of doxycycline (A), and from SUM-149 cells expressing control shRNA (CshRNA) or MUC1 shRNA (MUC1shRNA) (B). Pie charts depict the distribution of significantly altered alternative splicing events identified by rMATS, grouped as alternative 5′ splice site (A5SS, red), alternative 3′ splice site (A3SS, light green), mutually exclusive exons (MXE, green), intron retention (RI, blue), and skipped exons (SE, magenta). Values indicate the percentage of total differential events of each class. C. Validation of representative rMATS hits in BT-549/tet-MUC1shRNA cells. Boxplots show percent spliced in (PSI) values for an intron retention event in **INO80E** (left) and skipped-exon events in **CD44**(right) in doxycycline-negative (DoxNeg, blue) and doxycycline-treated (DoxPlus, orange) conditions. FDR values from rMATS are indicated above each comparison. Together, these data demonstrate that depletion of M1C alters the pattern of alternative splicing in TNBC cells, including intron retention and exon skipping events in genes linked to chromatin regulation and cell adhesion.*

M1C/MYC splicing axis as a targetable vulnerability in TNBC

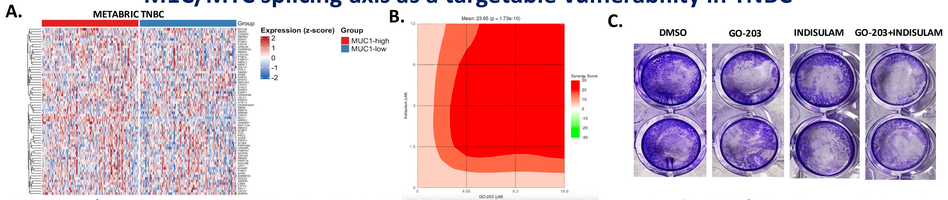


Figure 4. M1C/MYC splicing axis as a targetable vulnerability in TNBC- A. Heatmap of z-score–normalized expression of the MUC1/MYC splicing–associated gene signature in triple-negative breast cancers (TNBC) from the METABRIC cohort. Each column is a tumor sample and each row a signature gene. Tumors are grouped as MUC1-high (red) or MUC1-low (blue), showing preferential activation of the signature in MUC1-high tumors. B. Drug–drug synergy map for BT-549 cells treated with increasing doses of GO-203 (M1C inhibitor, x-axis) and indisulam (RNA-splicing modulator, y-axis). Red color indicates positive synergy scores, meaning the drug combination suppresses cell viability more than expected from the single agents. C. Representative crystal-violet colony-formation assay of BT-549 cells treated with DMSO, GO-203 alone, indisulam alone, or GO-203 plus indisulam. The marked reduction in colony number with the combination shows that co-targeting M1C and splicing more effectively impairs long-term clonogenic survival than either drug alone.

Discussion

Our findings identify M1C as a central driver of an oncogenic splicing program in triple-negative breast cancer (TNBC). In partnership with MYC, M1C upregulates core splicing regulators and remodels alternative splicing, including intron retention and exon skipping in genes involved in cell-cycle control, stress responses, and stemness. These M1C–dependent splicing changes support cancer stem cell properties providing a mechanistic link between M1C signaling and aggressive TNBC behavior. Our long-read and rMATS datasets also reveal numerous aberrant splice isoforms that may encode neoepitopes. As a future direction, we will use these datasets to computationally predict HLA-A2–binding peptides and identify candidate splice-derived neoantigens for functional testing. Overall, our results implicate the M1C/MYC–splicing axis as a critical regulator that couples tumor-intrinsic plasticity with a potentially targetable neoantigen landscape in high-risk TNBC.

Conclusions

M1C/MYC rewires splicing in TNBC- M1C, with MYC, reprograms alternative splicing, increasing intron retention and decreasing exon skipping in cancer-relevant genes, defining an oncogenic splicing program in TNBC.

Oncogenic splicing signature marks high-risk tumors- This M1C/MYC-associated splicing signature is enriched in MUC1-high tumors and correlates with more aggressive, poor-prognosis disease.

M1C/MYC splicing axis is targetable- Combined GO-203 and indisulam treatment synergistically suppress TNBC growth, defining this splicing axis as a targetable vulnerability.

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