



ABS N. 136

09 Monoclonal Gammopathies & Multiple Myeloma

NONO SHAPES MULTIPLE MYELOMA PROGRESSION THROUGH PARASPECKLE-DEPENDENT AND INDEPENDENT PATHWAYS

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INTRODUCTION: Multiple myeloma (MM) is an incurable cancer caused by malignant proliferation of bone marrow plasma cells. The lncRNA NEAT1, the scaffold of paraspeckle (PS), promotes MM progression by regulating DNA repair and cell survival. NONO, which stabilizes NEAT1 and supports PSs biogenesis, is upregulated in MM and associated with poor survival. In addition to its essential role within PSs, NONO may also have functions beyond PSs. This study aims to define how NONO shapes the transcriptomic landscape of MM plasma cells, both through its PS-associated and independent mechanisms.

METHODS: RNA was extracted from NONO-KD, NEAT1-KD, and scramble AMO1 and LP1 human MM cells lines (HMCL). RNA-seq libraries were prepared following Illumina Stranded TotalRNA PrepLigation with Ribo-zero Plus protocol (Illumina). Sequencing was performed on Illumina Novaseq 6000 S2 cartridge. CoMMpass data were retrieved from the Interim Analysis 15a (MMRF_CoMMpass_IA15a).

RESULTS: To investigate the role of NONO in both PS-related and independent pathways, we compared transcriptomic data from NONO-KD and NEAT1-KD AMO1 and LP1 cells. Overlapping pathways suggested PS-related functions. Silencing NEAT1 or NONO led to significant downregulation of gene sets involved in chromatin modification, as well as WNT/ β -catenin and NOTCH signalling pathways. Consistently, RNAseq analysis of NEAT1-overexpressing AMO1 cells revealed positive modulation of the same pathways. Further validation came from GSEA of CoMMpass samples stratified by NONO expression, comparing the two extreme quartiles.

Since NONO is essential for protecting NEAT1 from degradation, its silencing results in a marked downregulation of NEAT1 expression levels, thus impacting the transcriptome of NONO-KD cells in a NEAT1-dependent manner. As a result, all the pathways modulated in the NONO-KD HMCLs were also confirmed in the NEAT1-KD samples, making it impossible to identify any pathways regulated by NONO independently of PSs. However, the analysis of data from the extreme quartile of NONO in the CoMMpass dataset identified pathways absent in NEAT1-KD HMCLs, suggesting PS-independent roles for NONO in RNA processing, RNA trafficking, mitochondrial biogenesis, and cell-cell communication.

To validate pathways selectively regulated by NONO independently of PS, we focused on its role in intercellular communication and adhesion. Functionally, HUVECs cultured in conditioned media from NONO-KD AMO-1 or NCI-H929 cells showed disrupted VE-cadherin localization and expression, indicating that the NONO-dependent MM secretome can alter endothelial adhesion. These results reveal a potential PS-independent role for NONO in modulating the tumor microenvironment.

CONCLUSION: This study highlights the multifaceted transcriptional roles of NONO in MM, revealing both PS-dependent and -independent functions, and underscores its potential impact on tumor microenvironment regulation and disease progression.

Publicabile: SI, publicabile.