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LNCRNA NEAT1 ORCHESTRATES A CERNA NETWORK GOVERNING CORE PATHWAYS OF PROLIFERATION AND CELLULAR DYNAMICS IN MULTIPLE MYELOMA

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Background

The lncRNA NEAT1 is overexpressed in multiple myeloma (MM) plasma cells, playing a central role in disease pathogenesis by promoting MM progression. The competing endogenous RNA (ceRNA) network, defined by shared miRNAs among different transcripts, contributes to fine regulate several cellular processes. Perturbation of this network may unsettle the transcriptomic balance, potentially leading to pathological condition. While deregulation of NEAT1-driven ceRNA circuits are described in several cancers, evidence in MM are virtually absent.

Aims

To elucidate NEAT1 involvement in ceRNA network in MM.

Methods

RNAseq analysis was performed on NEAT1-KD AMO1, LP1, and NCI-H929 MM cell lines, and in NEAT1-transactivated AMO1 cells (AMO1-OVX). Differentially expressed transcripts were identified by LIMMA VOOM R package. StarBase 2.0 and TarBase databases were used to select miRNAs targeting NEAT1 and mRNAs. Statistical analyses were performed using the Wilcoxon rank-sum, Kruskal-Wallis, and Dunn tests. GSEA was performed on pre-ranked differentially expressed genes based on fold change. AMO-1 cells were electroporated with miRNA mimics (Life Technologies) by using the Neon Transfection System.

Results

We identified 41 putative target genes in the NEAT1-driven ceRNA network by selecting transcripts that were concurrently downregulated in NEAT1-KD AMO1 cells and upregulated in AMO1-OVX cells. We then defined a list of 97 miRNAs that can be sponged by NEAT1, target at least one of these 41 mRNAs, and exhibit appreciable expression levels in MM plasma cells (relative intensity levels $\log_2$>4 in at least 10 samples, GSE87830). Overall, we defined a ceRNA network comprising 1.146 edges and 139 nodes. To validate our results, we assessed the positive correlation between each candidate mRNAs and NEAT1 across available MM datasets (GSE116294, GSE13591, GSE6477, CoMMpass). 13 out of 41 targets showed a significant Spearman's correlation with NEAT1 in at least one dataset. Furthermore, all 41 targets exhibited expression trends similar to NEAT1 in NEAT1-KD LP1 and NCI-H929 cells, as well as in NEAT1-KD KMS27 cells (GSE178868), with 13 targets reaching statistical significance in at least one cell line.

To explore the functional implication of the ceRNA network, we performed functional enrichment analyses (EnrichR) of the 41 targets, identifying 78 significant gene sets related to cell cycle, senescence,

and RHO GTPase effectors. Notably, 17 gene sets were significantly enriched (-1.5≤ NES ≥1.5, padj <0.05) by GSEA in at least one experimental condition, including NEAT1-KD LP1, NCI-H929, and KMS27 cells, AMO1-OVX cells, or the extreme NEAT1 expression quartiles in the CoMMpass dataset. Importantly, gene sets related to cell cycle, senescence, and RHO GTPase effectors were validated in 5 out of 6 experimental conditions. Finally, *in vitro* experimental results obtained in AMO1 cells indicate that perturbations of specific miRNAs (miR-195, miR-106a-5p, and miR-106b-5p) in the ceRNA network lead to reduced expression of genes involved in the cell cycle pathway, including *KIF11*, *NSD2*, *PRKCA*, *ATAD2*, and *TPX2*.

Summary/Conclusion
Our study strongly suggests NEAT1 involvement in the ceRNA network in MM. Notably, through ceRNA-mediated mechanisms, NEAT1 appears to influence biological processes related to the cell cycle, in agreement with our previous published data showing that NEAT1-KD negatively affects proliferation and viability of MM cells.

Keywords: Cell cycle, Proliferation, Senescence, Multiple myeloma