Exploring the role of NONO in transcriptional regulation of cellular pathways in Multiple Myeloma: insights into its paraspeckle-dependent and independent regulatory mechanisms

Elisa Taiana[1], Andrea Devecchi[2], Ilaria Silvestris[3], Valentina Traini[3], Giuseppina Fabbiano[1], Marta Lionetti[3], Matteo Claudio Da Vià[1], Noemi Puccio[4], Ilaria Craparotta[5], Marco Bolis[5,6], Roberto Piva[7], Antonino Neri[8], Luca Agnelli[2,9], Francesco Passamonti[1,3], Niccolò Bolli[1,3], Domenica Ronchetti[3]

1 IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Hematology, Milan, Italy
2 Department of Diagnostic Innovation, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
3 University of Milan, Department of Oncology and Hemato-Oncology, Milan, Italy
4 Laboratory of Translational Research, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy
5 Computational Oncology Unit, Oncology Department, Mario Negri IRCCS, Milan, Italy
6 Bioinformatics Core Unit, Institute of Oncology Research (IOR), Bellinzona, Switzerland
7 Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy
8 Azienda USL-IRCCS di Reggio Emilia, Scientific Directorate, Reggio Emilia, Italy
9 Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy

BACKGROUND: Multiple myeloma (MM) is a malignant proliferation of bone marrow plasma cells (BMPCs) with variable clinical outcome, which, despite treatment advances, still remains incurable. LncRNA NEAT1, the scaffold of paraspeckle (PS) organelle, has been linked to cancer development and progression, by playing a critical role in DNA repair and cell survival in MM. NONO, a protein involved in NEAT1 stability and PS, is essential for several cellular functions and gene regulation. Notably, NONO is upregulated in MM patients, and its high expression correlates with poor OS and PFS. In addition to its essential role within PSs, NONO may also have independent functions in MM cells.

AIM: To dissect the role of NONO in modulating the transcriptomic landscape of MM plasma cells through next-generation sequencing, and elucidate its contribution both in relation to the PS and independently of it.

MATERIAL AND METHODS: RNA was extracted from NONO-KD, NEAT1-KD, and scramble AMO1 and LP1 cells. 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, accessed on 16 October 2020).

RESULTS: To explore the role of NONO in both PS-related and independent pathways, we compared data from NONO-KD or NEAT1-KD AMO1 and LP1 cells; overlapping pathways between NONO and NEAT1 should be suggestive of a NONO's involvement in PS-related functions.
In NEAT1 and NONO silenced cells we highlighted a significant downregulation of gene sets associated with chromatin modifications, as well as pathways related to WNT/β-catenin and NOTCH signalling. These findings were further confirmed by analyzing RNAseq data from AMO1 cells, which were previously engineered to overexpress NEAT1 and PSs. This analysis revealed a significant positive modulation of the same pathways (NES ≥ 1.5, padj < 0.05). Further confirmation was obtained by stratifying samples from the CoMMpass dataset based on NONO expression levels, comparing the expression profiles between the two extreme quartiles, and conducting GSEA on the list of differentially expressed coding genes.
Since NONO is essential for protecting NEAT1 from degradation, its silencing results in a marked downregulation of NEAT1 expression levels, thereby impacting the transcriptome of NONO-silenced cells in a NEAT1-dependent manner. As a result, all the pathways modulated in the NONO-KD MM cell lines (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 enabled the identification of specific pathways not shared with NEAT1-KD HMCLs, which

may suggest pathways that NONO regulates independently of PS in MM cells. This analysis revealed NONO's involvement in RNA splicing or maturation, cellular RNA trafficking to the cytoplasm, as well as its role in mitochondrial biogenesis and cell-matrix adhesion.

CONCLUSION: This study underscores the complex transcriptional roles of NONO in MM and its potential as a therapeutic target, particularly in pathways critical for disease progression. The in-silico validation further supports the clinical relevance of these findings, highlighting the value of the HMCLs model in advancing our understanding of MM.