P875 ACTIVATION OF LNCRNA NEAT1 LEADS TO SURVIVAL ADVANTAGE OF MULTIPLE MYELOMA CELLS BY SUPPORTING A REGULATORY LOOP WITH DNA REPAIR PROTEINS: RATIONAL BASES FOR NEAT1 THERAPEUTIC TARGETING IN THE DISEASE

Topic: 13. Myeloma and other monoclonal gammopathies - Biology & Translational Research

Elisa Taiana¹, Domenica Ronchetti¹, Vanessa Katia Favalesi¹, Ilaria Silvestris², Noemi Puccio¹, Katia Todoerti³, Cecilia Bandini⁵, Ilaria Craparotta⁷, Laura Di Rito⁷, Silvia Erratico⁸, Domenica Giannandrea¹⁰, Roberto Piva⁶, Marco Bolis⁷, Yvan Torrente⁹, Raffaella Chiaramonte¹⁰, Niccolò Bolli¹, Luca Baldini¹, Antonino Neri¹

Background:

Multiple myeloma (MM) is a fatal malignant proliferation of antibody-secreting bone marrow plasma cells characterized by marked genomic instability.

Long non-coding RNA (lncRNA) represents the largest class of non-protein coding genes in the human genome. Their crucial role in solid tumor and hematological malignances, including MM, is well documented.

NEAT1 is a highly expressed nuclear lncRNA, representing the core structural component of paraspeckle organelles. We previously demonstrated that NEAT1 silencing negatively regulates proliferation and viability of MM cells, both in vitro and in vivo, highlighting its pivotal role in DNA integrity maintenance, by regulating the homologous recombination. Despite the increasing information obtained by loss-of-function approaches, there is still a lack of information regarding possible oncogenic benefits acquired by MM cells upon NEAT1 overexpression.

Aims: Taking advantage of the use of the CRISPR/Cas9 SAM genome editing approach, the present study aimed to shed light on the biological and molecular implication of NEAT1 overexpression in MM.

Methods:

We adopted the CRISPR/Cas9 Synergistic Activation Mediator editing system to transactivate NEAT1 in AMO-1 MM cell line. LNA-gapmeR antisense oligonucleotide technology was used to silence NEAT1 in MM cells by gynmotic delivery. Hypoxia was induced by culturing the cells into a modular incubator chamber flushed with a mixture of 1% O₂, 5% CO₂ and 94%N₂ at 37 °C. NEAT1 expression was assessed by qRT-PCR and RNA-FISH. Cell cycle phases distribution and apoptotic cells percentage were assessed by flow cytometry. Clonogenic potential was evaluated by methylcellulose assay. Protein expression was investigated by WB and IF. Cell morphological analysis was performed after May-Grunwald Giemsa staining. Transcriptomic analysis was performed by RNA sequencing, following the TruSeq Stranded Total RNA protocol. Libraries with optimal quality and quantity were run on NextSeq 500. Statistical significance was determined by Student t test analysis; differences were considered significant when P-value was <0.05.

Results: We performed a transcriptomic analysis of AMO-1 cells either transactivated or silenced for NEAT1. Our data revealed a NEAT1 pivotal role in the maintenance of DNA integrity, showing a significant deregulation of almost all the crucial cellular DNA repair mechanisms for both single and double strand breaks. In particular, we...
found that NEAT1 transactivation affects DNA repair mechanisms through the activation of a molecular axis including two fundamental kinase proteins, i.e. ATM and DNA-PKcs, and the direct target pRPA32. Furthermore, our data strongly confirmed the activation of this NEAT1-orchestrated molecular axis whether MM cells are exposed to nutrient starvation and hypoxia, suggesting its implication in conferring oncogenic and pro-survival properties to MM cells. The disruption of this oncogenic loop leads to MM cell death and the loss of pro survival advantages, thus suggesting that NEAT1 should be considered a crucial factor for MM cells survival.

**Summary/Conclusion:** Overall, we provided novel important insights into the role of NEAT1 in MM pathobiology, demonstrating that its deregulation strongly correlates with adaptation to stress condition, often associated with late stages of the disease. Taken together, our results suggest that NEAT1 could represent Achilles' heel for MM cells and should be considered a potential therapeutic target for MM treatment.