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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Novel Combinatorial Therapeutic Options in NEAT1-Depleted Multiple Myeloma Cells By the Integration of in Vitro and in silico Drug Screening Approaches

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Multiple myeloma (MM) is an incurable plasma cells malignancy characterized by high genomic instability and clinical heterogeneity. Transcriptional landscape in MM is characterized by a marked de-regulation of long noncoding RNAs (lncRNAs) that influence disease progression and therapy resistance. We have shown that the lncRNA NEAT1 is overexpressed in MM patients and demonstrated that NEAT1 silencing negatively impact MM cells viability, both *in vitro* and *in vivo* suggesting that it may result in higher sensitivity to standard MM treatments. Herein, we adopted *in vitro* high throughput (HTS) and in *silico* drug screening approaches to explore the NEAT1 role in chemo-resistance scenario and identify novel putative therapeutic combination strategies.

For the HTS approach, a library of 320 selective inhibitors covering 123 targets involved in proliferative and survival pathways, was screened in NEAT1-KD AMO-1 MM cells and their relative scramble condition. Silencing of total NEAT1 was achieved through the gymnotic delivery of LNA-gapmeRs. Synergy score, calculated with Bliss analysis, identified 19 compounds having a synergistic effect, in combination with NEAT1 KD, leading to a decreased MM cells proliferation, compared to single treatments. These inhibitors span different cellular targets, grouped into five categories: cell cycle checkpoint, transcriptional regulators, protein tyrosine kinases, receptors channels, epigenetics regulators. Among the top scoring candidates, Aurora kinase A inhibitors were the most promising compounds able to individually exert a synergistic activity with total NEAT1-KD *in vitro*. Additionally, transcriptomic analysis was performed by bulk RNA sequencing in NEAT1-KD AMO-1 cell line. Libraries with optimal quality and quantity were run on NextSeq 500. Differential gene expression analysis identified 752 down-regulated genes and 957 up-regulated genes. This signature was used to query the Connectivity Map (CMap) database. Compounds mimicking NEAT1 KD transcriptional signature were selected with the highest connectivity score (> 90). Among the top scoring molecules, Aurora kinase A (AURKA) inhibitors were identified as able to mimic NEAT1 KD transcriptional signature.

Both approaches identified AURKA inhibitors as promising compounds able to affect NEAT1 functions. Based on this evidence, we started the validation of two different AURKA inhibitors on a panel of MM cell lines. Cell proliferation was assessed after 96 hours of NEAT1 silencing and 72 hours of drug treatment with Trypan-exclusion method and with live-cell imaging and analysis by taking advantage of Incucyte S3 ^{TM} technology. The synergy score was calculated with CompuSyn software. WB was used to assess the drugs on-target activity and qRT-PCR was used to validate the efficiency of NEAT1 KD. These data, suggest that NEAT1 KD and treatment with AURKA inhibitors can have similar biological outcome and can affect similar molecular pathways on MM cells. Overall, our results indicate that NEAT1 silencing combined with Aurora kinase A inhibition could represent a promising therapeutic option for MM patients, supporting the need to further characterize the molecular mechanisms underlying the synergistic effect observed in MM cells.

Disclosures No relevant conflicts of interest to declare.

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