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

Tumor Suppressive Mir-7 Targeting *PSME3* Improves the Efficacy of Histone Deacetylases Inhibitors in Multiple Myeloma

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Background: Histone deacetylases (HDACs) play a tumor-promoting role in multiple myeloma (MM), contributing to disease progression and treatment resistance. Panobinostat, an HDAC inhibitor, is clinicallyrelapsed or refractory MM. However, HDAC inhibitors have several limitations, such as drug resistance, poor tolerance, and limited use of combination drugs. Our previous comprehensive analysis of microRNAs modulated by HDAC inhibitors revealed new molecular pathogenesis and potential therapeutic targets in malignant lymphomas. However, the relationship between HDAC inhibitors and microRNAs in MM remains largely unknown.

Aim and methods: We treated the MM cell lines (MM.1S and KMS-11) with HDAC inhibitors (10 nM panobinostat and 1 μM vorinostat), collected RNA 48 h later, and conducted a comprehensive microarray analysisFurthermore, we explored the novel clinical impact of HDAC inhibitors via functional analysis of microRNAs.

Results: Microarray analysis showed that no microRNAs were commonly upregulated; however, only hsa-miR-7-5p (miR-7) was commonly downregulated in MM.1S and KMS-11 cells treated with both HDAC inhibitors. After exposing MM cell lines (MM.1S, KMS-11, RPMI-8226, H929, and U266) and patient samples to 10 nM panobinostat and 1 μM vorinostat for 48 h, we performed quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and observed significant downregulation in the expression of miR-7.

Next, we examined the function of miR-7 in MM by introducing miR-7 into MM cell lines. Introducing miR-7 significantly suppressed the proliferation of MM.1S and KMS-11 cells. We hypothesized that the introduction of miR-7 enhances the effects of HDAC inhibitors. We introduced miR-7 into MM.1S and cultured them for 48 h with panobinostat, followed by an apoptosis assay. The introduction of miR-7 significantly enhanced the apoptosis-inducing effect of panobinostat, suggesting a tumor-suppressive role of miR-7 in MM.

Furthermore, we examined the regulatory mechanisms of miR-7 in MM cells. Because it has been reported that MYC promotes miR-7 transcription, we focused on the role of MYC in miR-7 expression in MM. After exposing MM.1S and KMS-11 cells to HDAC inhibitors for 24 h, western blot analysis revealed that MYC was downregulated. After exposing MM.1S and KMS-11 cells to the BRD4 inhibitor JQ1, which suppresses MYC, at concentrations of 50 and 100 nM for 48 h, qRT-PCR showed that the expression of miR-7 was significantly downregulated in these cell lines. Similarly, introducing siRNA for MYC into MM.1S cells downregulated the expression of miR-7. These results suggest that HDAC inhibitors downregulate miR-7 expression by suppressing MYC expression. In addition, we examined the effect of bortezomib, a proteasome inhibitor, in combination with MM on the expression of miR-7. After exposing MM.1S, KMS-11, and H929 cells to 5 nM and 10 nM bortezomib for 48 h, we performed qRT-PCR and observed a significant upregulation in the expression of miR-7. Therefore, anti-myeloma effects of panobinostat by restoring miR-7 expression.

Finally, we investigated miR-7 targets that mediate the anti-myeloma effects. We introduced miR-7 into MM.1S cells via electroporation, collected RNA 48 h later, and performed microarray analysis. As a result, 26 genes were identified, and their expression was downregulated by less than one-third. Among these, four genes (*SLC6A9*, *LRRC59*, *EXOSC2*, and *PSME3*) possess conserved binding sites for miR-7. *PSME3* (proteasome activator subunit 3:PA28ϫ) has been reported as an oncogene in MM, and we focused on *PSME3*. Introducing miR-7 into MM.1S cells via electroporation significantly suppressed *PSME3* expression. Furthermore, when MM.1S cells were exposed to HDAC inhibitors, *PSME3* expression was significantly upregu-

lated. It has been reported that the inhibition of PSME3 increases sensitivity to proteasome inhibitors in MM. The favorable combination effect of bortezomib with HDAC inhibitors could be due to the modulation of the miR-7- *PSME3* axis.
Conclusion: We revealed that miR-7 exerts anti-myeloma effects and that *PSME3* is one of the targets of miR-7. miR-7 is an interesting microRNA because it is positively regulated by MYC, even though it is tumor-suppressive. Elucidating the role of miR-7 may lead to the re-discovery of HDAC inhibitors in MM therapeutic strategies.

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