1. BIOLOGY AND PRECLINICAL

ZEBULARINE MODULATES DNA METHYLTRANSFERASE EXPRESSION THROUGH ASSOCIATED HISTONE COMPLEXES AT THEIR PROMOTERS IN MULTIPLE MYELOMA CELL LINES

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Background. DNA methylation is a key epigenetic mechanism that influences gene regulation and cellular function. This process is mediated by DNA methyltransferases, enzymes that catalyze the transfer of methyl groups onto cytosine of DNA. Dysregulation of DNMT function and abnormal DNA methylation patterns are frequently observed in cancer and contribute to tumor development and progression. Zebularine is a nucleoside analog of cytidine that inhibits DNA methyltransferase activity through the formation of a covalent enzyme–DNA complex.

Objective. This study aimed to investigate the effect of zebularine on DNA methyltransferase gene expression and epigenetic modifications at their promoter regions in multiple myeloma cell lines.

Materials and Methods. DNA was isolated from 3 myeloma cell lines (KMS-12-BM, KMS-12-PE, U266) treated with the 10 µM, 100 µM, 200 µM, and 250 µM zebularine for DNMTs expression. The expression of DNMTs genes was quantified by RT-PCR using primers specific for both promoter or coding regions of the respective DNMT. For chromatin immunoprecipitation (ChIP) analysis, cells were treated with 250 µM zebularine, and chromatin was immunoprecipitated using antibodies against H3K9me3, H3K36me2, H3K36me3, and H3K4me3. The enrichment of these histone modifications at DNMT1 and DNMT3B promoters was quantified by RT-PCR using promoter-specific primers.

Results. The expression of DNMT1 in KMS-12-PE and KMS-12-BM cell lines was decreased after 250 µM zebularine treatment, with a statistically significant difference at $p < 0.05$ (p-value 0.0024 for KMS-12-PE and 0.0011 for KMS-12-BM). Similarly, DNMT3B expression was significantly reduced in both KMS-12-PE and KMS-12-BM cells ($p < 0.05$, p-value 0.002 for KMS-12-PE and 0.0001 for KMS-12-BM) after 250 µM zebularine treatment. In KMS-12-PE cells, 250 µM zebularine treatment was associated with increased enrichment of repressive histone marks H3K9me3 and H3K36me2 at the promoters of DNMT1 and DNMT3B, accompanied by reduced enrichment of active marks H3K36me3 at both promoters and H3K4me3 at the DNMT1 promoter. Similarly, in KMS-12-BM cells, zebularine induced an increase in repressive H3K36me2 marks at the promoters of DNMT1 and DNMT3B and H3K9me3 at the DNMT3B promoter, along with a decrease in the active H3K36me3 mark at both promoters.

Conclusion. Zebularine treatment significantly reduced DNMT1 and DNMT3B expression in KMS-12-PE and KMS-12-BM cell lines and was associated with alterations in the chromatin state of their promoters, characterized by changes in repressive and active histone complexes, with the potential to inactivate gene transcription.

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