

Epigenetic regulation of nuclear PLCbeta1 and Cyclin D3 during Azacitidine treatment

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The Myelodysplastic Syndromes (MDS) are a heterogeneous group of bone marrow disorders characterized by alterations of the hematopoietic stem cells that lead to anemia, neutropenia, bleeding problems and infections. The evidence of a clinical correlation between the presence of a monoallelic gene deletion of Phospholipase C β 1 (PLC β 1) and the progression of MDS to Acute Myeloid Leukemia (AML) opened new perspectives of research and treatments. Patients affected by MDS with a higher risk of AML evolution have a reduction in the expression of the nuclear PLC β 1, which is also epigenetically relevant in MDS. This strengthens the importance of PLC β 1 localization. In fact, PLC β 1 is a molecular target for hypomethylating agents, such Azacitidine (AZA)(1). High-risk MDS patients that respond to the drug showed an increased expression of nuclear PLC β 1 and its downstream target Cyclin D3 (CCND3), an induction of normal myeloid differentiation, and a better prognosis. Stemming from these data, our goal was to analyze the correlation between CCND3, PLC β 1 and AZA treatment. Firstly, we treated two different cellular lines, AML HL60 and histiocytic lymphoma U937, with AZA 5 μ M (Ec50 for HL60 cells) for 24 hours. Then, we used Real-Time PCR and Western blot to quantify both gene and protein expression. Moreover, we showed that CCND3 promoter has one CpG island. For this reason, it is possible that AZA could directly affect both PLC β 1 and CCND3 promoters. Therefore, we studied PLC β 1 binding to CCND3 promoter by chromatin immunoprecipitation (CHIP), before and after AZA treatment. Our results evidenced that the recruitment of PLC β 1 to CCND3 promoter is specifically increased after AZA treatment, leading to suppose that PLC β 1 could have a pivotal role in MDS with either a direct or indirect effect on cell cycle, proliferation and differentiation. These complicate relations need future deepening in order to demonstrate how PLC β 1 binding actually regulates CCND3 expression and how much this expression depends on CCND3 direct promoter demethylation and PLC β 1 control.

References

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Keywords

Nucleus; phospholipase C β 1; azacitidine; myelodysplastic syndromes; cyclin D3.