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Role of Nuclear PI-PLCbeta 1 during Azacitidine-induced Myeloid Differentiation

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Phospholipase Cbeta1 (PI-PLCbeta1) 1 is a key enzyme of the phospholipase family, a class of molecules involved in the lipidic cell signaling that play central roles in cell proliferation and differentiation. PI-PLCbeta 1 has two splicing variants: 1a, with cytoplasmatic and nuclear localization, and and 1b, which is mainly nuclear.

Myelodysplastic syndromes (MDS) are a heterogeneus group of hematological diseases characterized by a stem cell defect that causes anemia, cytopenia and leucopenia.

MDS can evolve into acute myeloid leukemia (AML). In patients in which the probability is higher, called high risk MDS, the treatment aims to delay the tumor progression and restore the hematopoietic differentiatin.

As the most frequent alterations in high risk MDS cells that cause AML evolution are of epigenetic nature, the therapeutic approach for high risk MDS is based on demethylating agents, like azacitidine. [1] Furthermore, there is a statistical significant increase in the levels of expression of nuclear PI-PLCbeta1 in MDS patients responding to azacitidine [2].

Stemming from these data, here we further analyzed the involvement of PI-PLCbeta1 at different stages of azacitidine-induced hematopoietic differentiation, with particular reference to the myeloid lineage.

Due to the limits of a heterogeneous population in patient blood samples, we firstly studied the azacitidine effect on AML cell lines, using cells at different stages of hematopoiesis. First, by enzymatic method, we showed that azacitidine induces PI-PLbeta1 demethylation. Then, by flow cytometry, we demonstrated not only that azacitidine affects cell cycle and apoptosis, but also that it changes the expression of specific surface myeloid markers. Subsequently, we studied the azacitidine effect on nuclear PI-PLCbeta1 and other enzymes involved in phosphoinositide signaling, firstly by Real Time PCR, then by Western Blotting and immunocytochemistry analyses, showing that azacitidine can specifically change the expression and localization of specific inositide players.

All in all, our results show that azacitidine induces a myeloid differentiation in AML cell lines, particularly rapid in those already showing a myeloid committment (e.g. HL60 promyelocytic cells), and a late one in cells with a higher stem cell population (i.e. KG1 AML cells). More importantly, we demonstrated that it is the nuclear isoform of PI-PLCbeta1 to play a pivotal role in myeloid induction, particularly in the first phases of differentiation.

References

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Key words

Nuclear PI-PLCbeta 1, Myeloid Differentiation, Cellular Localization.