NEDDylation effect on MMP-9 expression in a model of Chronic Lymphocytic Leukemia

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INTRODUCTION

Chronic Lymphocytic Leukemia (CLL) is a malignant disease caused by the accumulation of mature B lymphocytes due to an inherent defect in apoptosis. This disease is more common in the West countries and it is diagnosed in an advanced age, between 70 and 72 years old. Also, it affects twice more men than women. CLL has variable clinical manifestations because it could be very aggressive or indolent for some years (1). One of the most important characteristics of LLC is the constitutive activation of some signaling pathways such as NF-kB and AP-1, which enhance cell survival. MMP-9 plays an important role in cell survival, angiogenesis and metastasis (3). MMP-9 promoter contains several binding sites for NF-kB and NF-1 transcription factors. The last one mediates p38 induced upregulation of MMP-9(2). On the other hand, NF-kB induces the expression of apoptosis inhibitors such as Xiap and Bcl-2 family, Furthermore, NF-kB activates the orphan receptor NURR1 that has been reported to impair MMP-9 induction by IL-1ß. These signaling pathways are closely linked to the capacity of invasion and metastasis in cancer cells(4).

Previous studies from our group described that an inhibition of neddylation with MLN4924 in CLL cells represses the expression of MMP9 in parallel with an increase in apoptosis. NEDDylation is involved in proteins homeostasis and some of its target proteins have important roles in cell cycle progression and survival in cancer(5). Additionally, studies demostrated that MLN4924 may have activity against tumors that are dependent on NF-κB signaling for survival (6). Therefore, we focused our study on the effect of this inhibitor in the expression of MMP-9 and how it is regulated by NF-kB and AP-1 pathways in MEC-1, a cell line of Chronic Lymphocytic Leukemia.

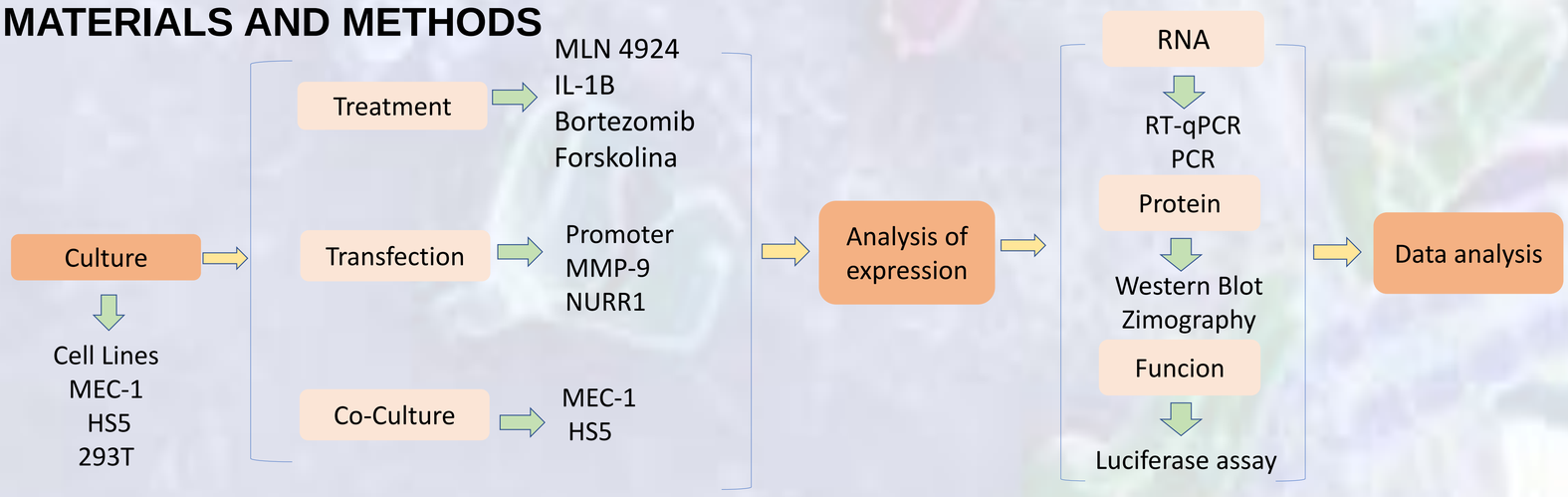
OBJECTIVE:

To study the molecular mechanisms by which NEDDylation modulates the expression of MMP9, using the cell line MEC-1 as a model of Chronic Lymphatic Leukemia.

HYPOTHESIS:

Previous data in the laboratory shows that inhibition of NEDDylation by MLN4924 reduces the expression of MMP-9 messenger in the primary cells of patients with Chronic Lymphatic Leukemia (CLL). We hypothesize that MLN4924 may be affecting NF-kB or AP-1 signaling pathways.

MATERIALS AND METHODS



RESULTS:

Effect of MLN4924 on cell viability

7-AAD and Anexinn labeling analyzed by Flow cytometry after treatment with MLN4924 showed a dose-dependent induction of apoptosis, but not with LY294002.

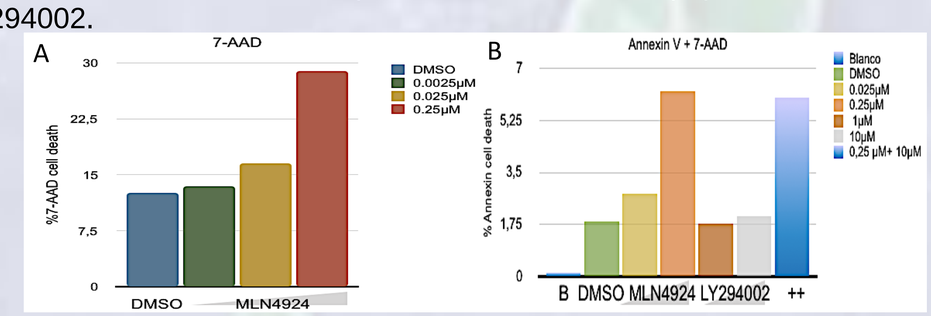


Figure 1: A) Apoptosis after MLN4924 treatment on MEC-1 cells. B) Late apoptosis after treatment with MLN4924 and LY294002 on MEC-1 cells.

Effect of MLN4924 on signaling pathways on MMP-9

NF-kB: IL-1ß has an effect on the expression of Bcl-2 but not on MMP-9. Furthermore, Bortezomib has an dose-dependent effect on MMP-9 overexpression.

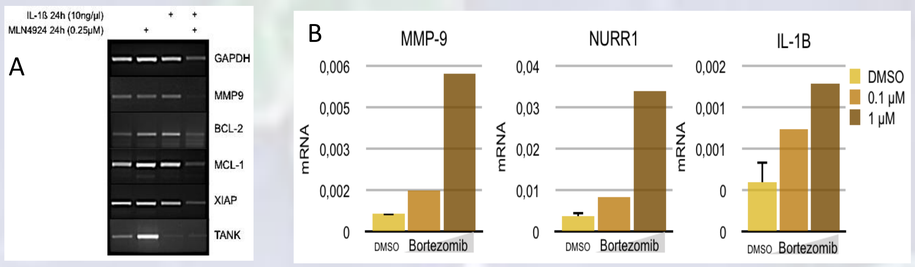


Figure 4: A) Analysis of MMP-9 expression and NF-kB genes after IL-1ß and MLN4924 treatment on MEC-1 cells. B) Analysis of MMP-9, NURR1 and IL-1ß after Bortezomib treatment on MEC-1.

AP-1: p38 and ERK2 repress the basal activity of MMP-9 (-250/+22), and they block the inhibitory effect of MLN4924. The regulatory region between positions -941 and +86 of NURR1 promoter was induced by p38, but above all of ERK2. Inhibition of NEDDylation reversed the effect of p38 but potentiated that of ERK2.

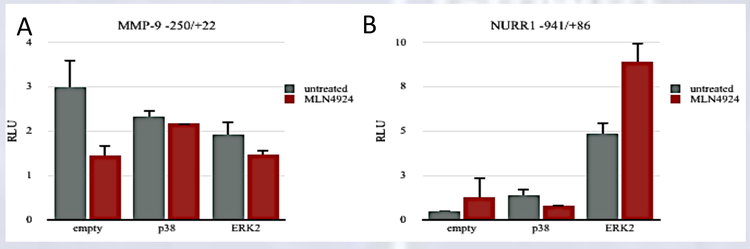


Figure 5: A) Expression of MMP-9 promoter and the effect of MLN4924 on 293T cells. B) Expression of NURR1 promoter and effect on 293T cells.

PKA y NURR1: Forskolin induces the expression of MMP-9 and NURR1 on MEC-1 and B-CLL cells. Moreover, Forskolin and MLN4924 show a synergistic effect on the transcription of NURR1, but not on MMP-9.

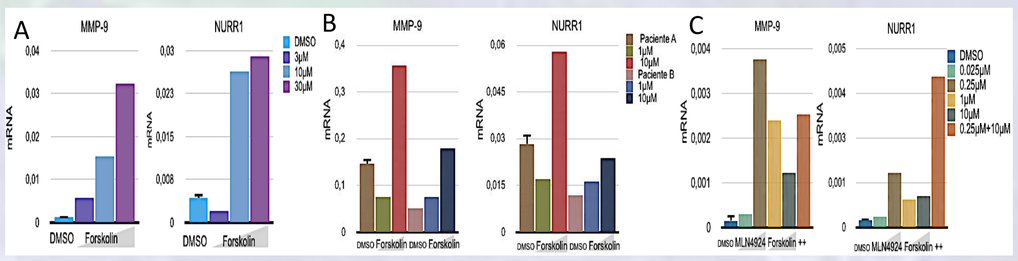


Figure 6: A) Expression of MMP-9 and NURR1 after treatment with Forskolin on MEC-1 cells. B) Expression of MMP-9 and NURR1 after treatment with Forskolin on B-LLC cells. C) Expression of MMP-9 and NURR1 after treatment with MLN4924 and Forskolin on MEC-1 cells.

Effect of MLN4924 on gen expression in MEC-1

MMP-9, NURR1 and genes regulated by NF-kB (BCL-2, MCL-1, TANK) were overexpressed at mRNA level with the increment of MLN4924 (24h). Moreover, the activity of MMP-9 in the culture medium was slightly increased by the treatment with MLN4924 (40h).

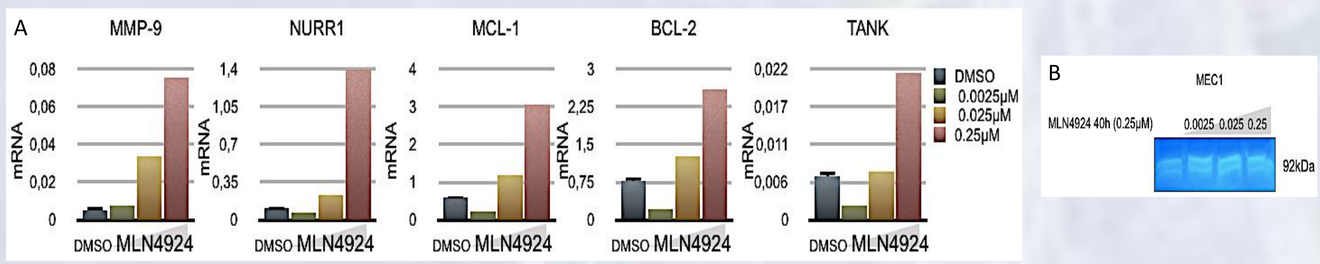


Figure 2: A) Expression of MMP-9, NURR1, MCL-1, BCL2, TANK after treatment with MLN4924 in MEC-1. B) Analysis of MMP-9 protein after treatment with MLN4924 in MEC-1 by Zimography.

MLN4924 reverses the effect of LY294002 on MMP-9 expression, and the same effect is observed over MCL-1, TANK, BCL-XL and XIAP. However, NURR1 has an opposite behaviour, with Ly294002 potentiating the effect of MLN4924. No effect was observed over BCL-2.

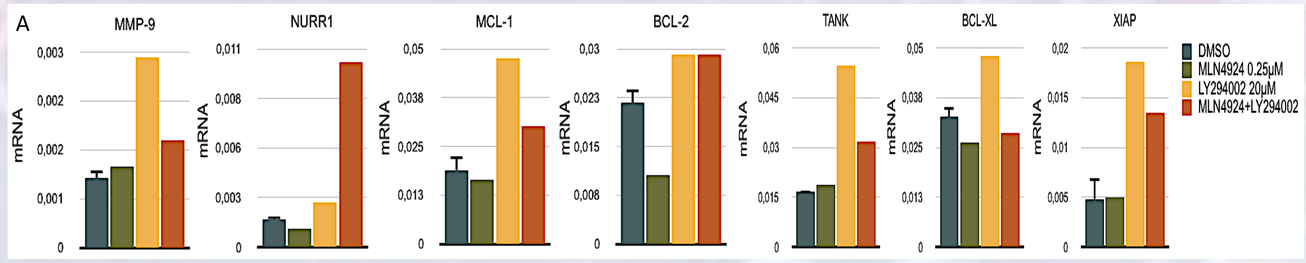


Figure 3: A) Expression of MMP-9, NURR1, MCL-1, BCL2, TANK after treatment with MLN4924 in MEC-1.

Stromal intervention on MEC-1 cells

The co-culture with HS-5 cells induced the expression of MMP-9 in MEC-1 cells. However, the same did not happen with the NURR1. Bortezomib and MLN4924 reversed the induction of MMP9 in coculture with HS-5 cells.

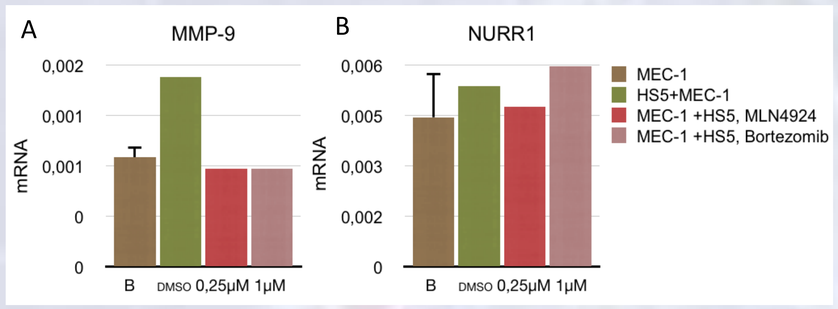


Figure 7: A) Expression of MMP-9 after treatment with MLN4924 and Bortezomib in the co-culture MEC-1 and HS5. B) Expression of NURR1 after treatment with MLN4924 and Bortezomib in the co-culture MEC-1 and HS5.

Conclusions:

MLN4924, Ly294002, Bortezomib, IL-1ß and Forskolin increase the expression of MMP-9 in MEC-1 cells, showing a possible proapoptotic feature of MMP-9 in these cells.

Coexpression of MCL-1, BCL-2, NURR1, TANK, BCL-XL and XIAP as well as MMP-9, suggest that NF-kB and AP1 could be involved in the modulation of MMP-9 in MEC-1 cells .

The co-culture of MEC-1 cells with stromal cells (HS5) induces the expression of MMP-9. Treatment with MLN4924 or Bortezomib reverse this effect over MMP-9, suggesting a role of NF-kB pathway in this induction

The data obtained in the MEC-1 cell line regarding the modulation of MMP-9 expression by NEDDylation contrast with those previously obtained in primary B-CLL lymphocytes. Presumably, the promyelocytic transformation from which they are derived has affected these processes, so we concluded that they are not a good model of LLC in this respect.

References:

1) Motta, M., Wierda, W., Ferrajoli, A. (2009). Chronic lymphocytic leukemia. Cancer, 115(17), 3830–3841. DOI:10.1002/cncr.24479. 2) Ringshausen, I., Dechow, T., Schneller, F., Weick, K., Oelsner, M., Peschel, C., & Decker, T. (2004). Constitutive activation of the MAPkinase p38 is critical for MMP-9 production and survival of B-CLL cells on bone marrow stromal cells. Leukemia, 18(12), 1964–1970. DOI:10.1038/sj.leu.2403544. 3) Kipps, T., Stevenson, F., Wu, C., Croce, C., Packham, G., Wierda, W., O'Brien, S., Gribben, J., Rai, K. (2017). Chronic lymphocytic leukaemia. Nature Reviews Disease Primers, 3, 16096. DOI:10.1038/nrdp.2016.96. 4) Vladimirova, R., Popova, D., Vikentieva, E., Guenova, M. (2015). Chronic Lymphocytic Leukemia -Microenvironment and B Cells. Intech, 9, 247-276. DOI:10.5772/60761. 5) Xirodimas, D. (2008). Novel substrates and functions for the ubiquitin-like molecule NEDD8. Biochemical Society Transactions, 36(5), 802-806. DOI:10.1042/BST0360802. 6) Godbersen, J., Paiva, C., Danilova, O., Berger, A., Brown, J., & Danilov, A. (2015). Targeting neddylation effectively antagonizes nuclear factor-κB in chronic lymphocytic leukemia B-cells. Leukemia & Lymphoma, 56(5), 1566–1569. DOI:10.3109/10428194.2014.990901.