The RNA-binding protein Musashi2 is regulated by NOTCH1/KLF4 pathway and support tumor survival keeping CLL cells in proliferative niches during disease progression

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BACKGROUND

Post-transcriptional regulation is an essential cellular mechanism controlling gene expression. RNA-binding proteins (RBPs) regulates the fate of RNA molecules, and their dysregulation can lead to cancer. In chronic lymphocytic leukemia (CLL), high levels of the RBP Musashi2 (MSI2) have been linked to tumor cell survival and poor prognosis (Palacios et al., Leukemia, 2021).

The increase in CLL-cells in patients stems from a small fraction of dividing CD5$^+$B cells. This proliferative fraction (PF) directly correlates with poor outcomes making it an important target for therapy. We observed elevated MSI2 levels in CLL compared to healthy donor (HD) B cells, particularly within the PF. Targeting MSI2 or its function effectively eliminates CLL cells, suggesting MSI2 or its regulatory molecules could be responsible for the clinical course of CLL patients. Therefore we studied: **1-** how MSI2 overexpression is induced in CLL-B cells, and **2-** MSI2's role in dividing CLL cells.

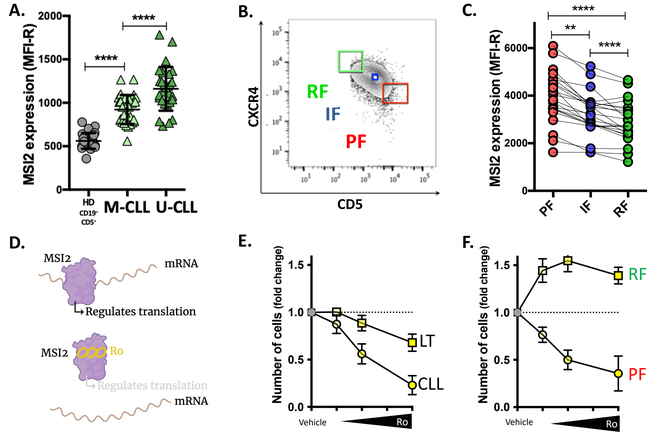
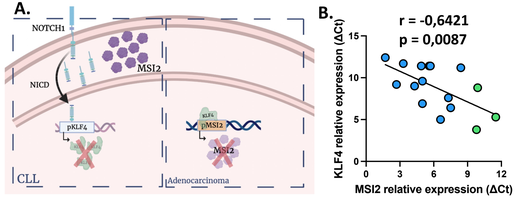


Figure 1: Musashi2 is associated with poor prognosis and tumor cell survival. A. MSI2 expression in B cells (CD19$^+$CD5$^+$) from M-CLL and U-CLL patients (light green and dark green triangles) compared to HD B cells (CD19$^+$CD5$^+$, grey circles). **B.** Representative flow cytometry dot plot of the CLL B-cells and its fractions; resting, intermediate and proliferative (green, blue and red respectively). **C.** MSI2 expression in the different CLL B-cells fractions (red, blue and green circles) **D.** Graphic representation of MSI2 function in the cells. **E.** Viable B and autologous T cells treated with 5, 10, and 20μM of Ro 08-2750 **G.** Viable relative PF (left) and RF (right) cells after treatment with Ro 08-2750.

RESULTS

1- Is the absence of KLF4 regulated by NOTCH1, responsible for MSI2 overexpression in CLL?



NOTCH1 ⊣ KLF4 ⊣ MSI2

Figure 3: Model of NOTCH1/KLF4 pathway on MSI2 expression in CLL. A. Graphic representation of the molecular mechanism that could be involved in MSI2 overexpression. **B.** Correlation between KLF4 and MSI2 relative expression (ΔCT) in CLL and HD B cells.

2- NOTCH1/KLF4 pathway regulates MSI2 expression in progressor CLL patients

NOTCH1 ⊣ KLF4 ↑ ⊣ MSI2 ↓

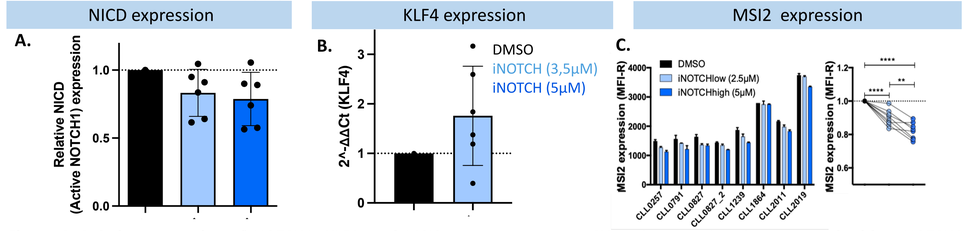


Figure 4: Relative expression of NOTCH1, KLF4, and MSI2 among progressor CLL cells treated and not treated with a NOTCH1 inhibitor (γ-secretase, iNOTCH). A NICD protein expression levels (MFI) in treated (3,5 and 5,0μM, light blue and blue respectively) and non treated (black) CLL cells by flow cytometry. **B.** KLF4 expression on treated (3,5μM) and not treated cells by RT-PCR. **C.** Relative MSI2 expression on treated (2,5 and 5,0μM, light blue and blue respectively) not treated cells (black) by FC.

3- KLF4 binds to MSI2 promoter in CLL B cells and it is regulated by NOTCH1

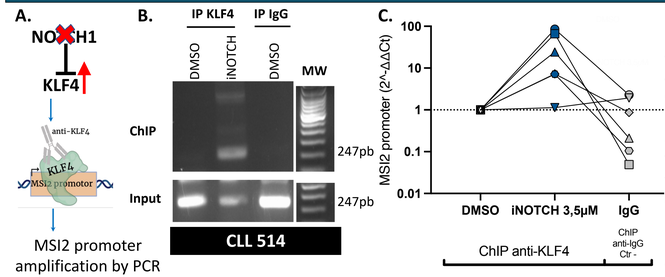
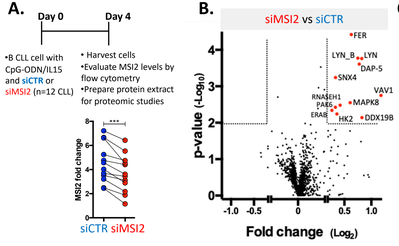


Figure 5: KLF4 immunoprecipitation on primary CLL cells treated with (iNOTCH) and without (DMSO) NOTCH1 inhibitor. A. Workflow strategy. **B.** Semi-quantitative PCR analysis of KLF4 immunoprecipitation depicted in 2% agarose gel electrophoresis. Cells were treated with vehicle (DMSO) and iNOTCH for KLF4 ChIP. DMSO+IgG was used as control of the ChIP. **C.** MSI2 promoter levels after the ChIP for not treated (white), treated (blue) and control (grey) conditions.

4- MSI2 inhibit cytoskeleton rearrangement in activated CLL cells



Target Full Name	Entrez Gene	FC	p-value	Function
Tyrosine-protein kinase Fer	FER	1.5	3.8E-05	Cell migration / cytoskeleton rearrangment
Tyrosine-protein kinase Lyn, isoform B	LYNB	1.7	0.00017	
Tyrosine-protein kinase Lyn	LYN	1.8	0.00017	
Proto-oncogene vav	VAV	2.3	0.00177	
Mitogen-activated protein kinase 8	MK08	1.5	0.0028	
Serine/threonine-protein kinase PAK 6	PAK6	1.3	0.00377	
Death-associated protein 5	DAP-5	1.7	0.00025	Apoptosis and DNA repair
Ribonuclease H1	RNASEH1	1.3	0.00329	
Sorting nexin-4	SNX4	1.3	0.00057	Intracelular trafficking
ATP-dependent RNA helicase DDX19B	DDX19B	1.8	0.00721	
3-hydroxyacyl-CoA dehydrogenase type-2	ERAB	1.2	0.00459	Cell metabolism
HexoKinase-2	HK2	1.3	0.00576	

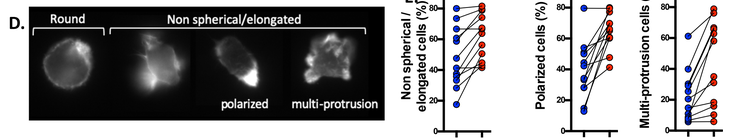
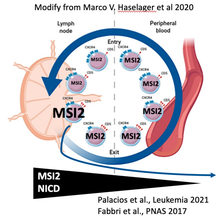


Figure 2: MSI2 regulate cell migration in activated CLL B cells. A. Cells from 12 patients were treated 4 days with CpGODN+IL15 and siCTR or siMSI2 *in vitro*. After eliminating dead cells and determining MSI2 levels by flow cytometry, protein extracts were prepared for proteome analysis. **B.** Volcano plot illustrating the differential protein levels identified after the proteome analysis of CLL B cells with and without MSI knock-down. **C**. Ingenuity Pathway Analysis indicates that the proteins differentially express associate with migration, apoptosis, intracellular trafficking, and cell metabolism **D.** Effect on cell morphology as a consequence of cytoskeleton remodeling after MSI2 knockdown. **E.** Percentage of elongated, polarized and multi-protruded cells in siMSI2 vs siCTR treated cells.

SUMMERY



- NOTCH1/KLF4 might regulate MSI2 expression in CLL cells
- KLF4 negatively regulates MSI2 expression through NOTCH1 pathway
- MSI2 inhibit cytoskeleton rearrangement in activated cells

High levels of MSI2 in activated cells inhibit cell migration which might contribute to disease progression keeping the cells in proliferative niches.

Thus, we propose that understanding the role of MSI2 in CLL B cells will provide clues to the mechanisms involved in leukemia-cell proliferation and disease progression.