#1018 Unraveling the NOTCH1/MSI2/c-MYC Signaling Pathway Reveals a Novel Vulnerability in Chronic Lymphocytic Leukemia Progression

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BACKGROUND

Gene expression regulation is vital for cellular homeostasis, and its dysregulation is associated with various types of cancers. Therefore, understanding its role in cancer could give us tools for identifying new therapeutic targets. At the post-transcriptional level, RNA-binding proteins are crucial in controlling geneexpression by regulating the temporal, spatial, and functional dynamics of messengers RNAs (mRNA). In chronic lymphocytic leukemia (CLL), our group has described high levels of the RNA-binding protein Musashi2 (MSI2) associated with tumor cell survival and poor prognosis (Palacios et al., Leukemia, 2021). Notably, reducing MSI2 levels or inhibiting its function eliminates both human and murine CLL cells. Given that MSI2 regulates cancer-associated biological processes, MSI2 signaling pathways that induce MSI2 overexpression, its role in CLL cell proliferation or its target mRNAs represent promising novel therapeutic targets.

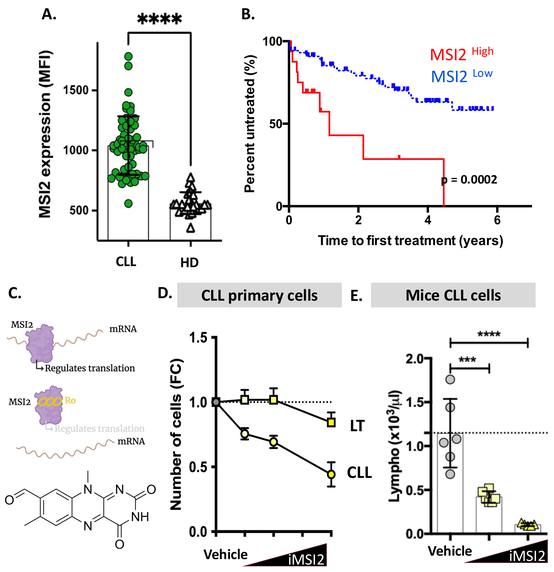


Figure 1: Musashi2 is associated with poor prognosis and tumor cell survival. A. MSI2 relative expression in B cells from CLL patients (green circles) and healthy donors (triangle). **B.** Kaplan–Meier curves for time-to-first-treatment (TTFT) in CLL patients with higher or lower MSI2 mRNA levels. **C.** Graphic representation of MSI2 function in the cells. **D.** Viable B and autologous T cells treated with 5, 10, and 20µM of iMSI2. **E.** Mice CLL cells (B220⁺CD5⁺) treated with vehicle (grey) and iMSI2 (yellow).

RESULTS

1- KLF4 regulates MSI2 expression in CLL B cells through NOTCH1 signaling

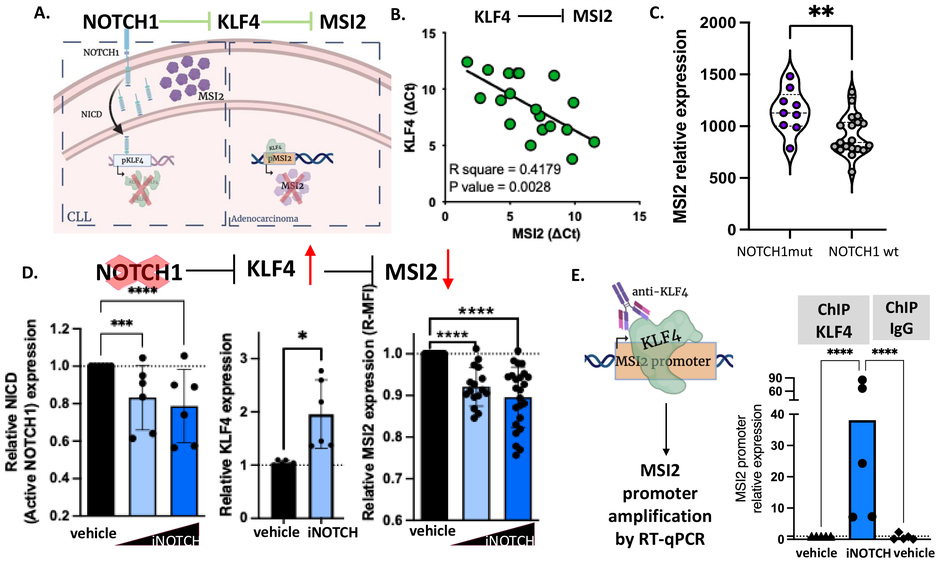


Figure 2: NOTCH1/KLF4 pathway regulates MSI2 expression in progressor CLL patients A. Graphic representation of the molecular mechanism that could be involved in MSI2 overexpression. **B.** Correlation between KLF4 and MSI2 relative expression (ΔCT) in CLL cells. **C.** MSI2 expression in CLL cells with mutated (Violet) and non-mutated NOTCH1 (Grey). **D.** Relative expression of active NOTCH1 (NICD), KLF4, and MSI2 among progressor CLL cells treated and not treated with a NOTCH1 inhibitor (iNOTCH). NICD protein expression levels (MFI) in treated (3,5 and 5,0µM, light blue and blue respectively) and non treated (black) CLL cells by FC. KLF4 expression on treated (3,5µM) and non treated cells by RT-PCR. Relative MSI2 expression on treated (3,5 and 5,0µM, light blue and blue respectively) and non treated cells (black) by FC. **E.** KLF4 immunoprecipitation on primary CLL cells treated with iNOTCH or the vehicle. MSI2 promoter levels after the ChIP for non treated (white), treated (blue) and IgG control (grey) conditions.

2- MSI2 regulates cytoskeleton rearrangement/cell migration in activated CLL B cells

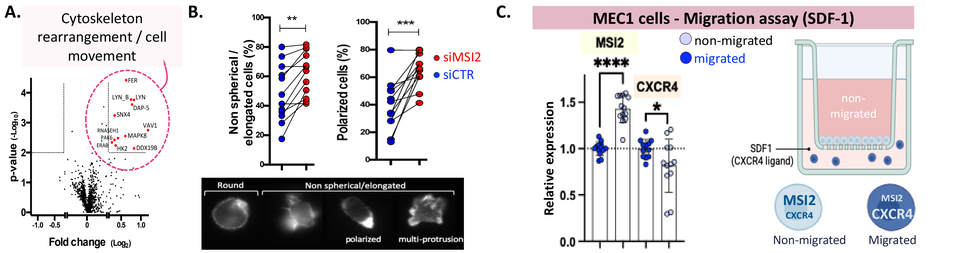


Figure 3: MSI2 regulates cell migration in activated CLL B cells. A. Volcano plot illustrating the differential protein levels identified after the proteome analysis of CLL B cells with and without MSI2 knock-down. **B.** Percentage of non spherical/elongated, polarized cells in siMSI2 vs siCTR treated cells. Effect on cell morphology as a consequence of cytoskeleton remodeling after MSI2 knockdown. **C.** Relative expression of MSI2 and CXCR4 on migrated (Blue) and non-migrated (Grey) MEC1 cells after a migration assay towards CXCR4 ligand (SDF1).

3- MSI2 regulates c-MYC expression in the tumor clones of CLL patients

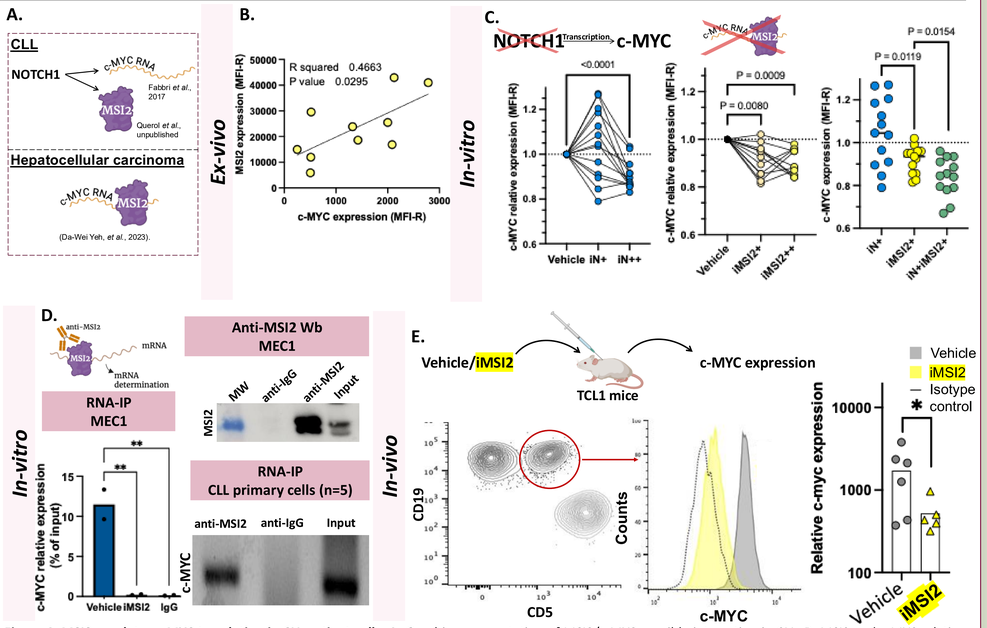
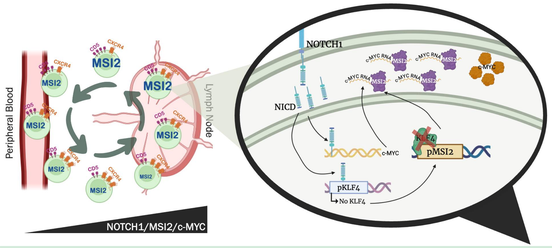


Figure 4: MSI2 regulates c-MYC translation in CLL patient cells. A. Graphic representation of MSI2/c-MYC possible interaction in CLL. **B.** MSI2 and c-MYC relative expression (MFI-R) correlation in CLL B cells. **C.** c-MYC relative expression in treated with iNOTCH (3,5 and 5 µM) CLL patient cells (left panel). c-MYC relative expression in treated with iMSI2 (5 and 10 µM, light yellow and yellow respectively) CLL patient cells (middle panel). c-MYC relative expression in treated with iNOTCH, iMSI2 and both inhibitors (blue, yellow and green respectively) CLL patient cells (right panel). **D.** RNA Immunoprecipitation workflow strategy. Western blot of the immunoprecipitation complex (RNA-protein) using anti-MSI2 or anti-IgG (negative control) with anti-MSI2 in MEC1 cell line (upper panel). RT-qPCR analysis of c-MYC immunoprecipitated in MEC1 cell line. Semi-quantitative PCR analysis of c-MYC immunoprecipitated depicted in 2% agarose gel electrophoresis (lower panel) of CLL patients. **E.** Gate strategy of the CLL cells CD19⁺CD5⁺ in the TCL1 mice (left panel). Representative histogram showing c-MYC protein levels in CLL cells treated with iMSI2 (yellow) or the vehicle (grey) (middle panel). Relative c-MYC expression in treated with iMSI2 (yellow) or the vehicle (grey) (right panel).

SUMMARY



- ✓ **NOTCH1 induces MSI2 expression**
- ✓ **NOTCH1 induces c-MYC transcription** (Fabbri et al., 2017)
- ✓ **MSI2 binds to c-MYC mRNA and regulates its translation**

NOTCH1/MSI2/C-MYC signaling axis contributes to the proliferation of the leukemic B cells from CLL patients.