Activation of S100A9/EMMPRIN axis triggers survival/proliferation pathways in leukemic cells
A novel target for Chronic Lymphocytic Leukemia

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BACKGROUND

The microenvironment has emerged as a crucial contributor to the pathogenesis of CLL, with cell-cell interactions and soluble molecules signaling activating pathways leading to disease progression and therapy resistance. Our group previously identified the presence of exosomes carrying the pro-inflammatory protein S100A9 in progressive patients, along with an overexpression of its receptor EMMPRIN in CLL B cells. In this new approach, we identified the S100A9/EMMPRIN axis a previously undescribed pathway in CLL. This axis may contribute to tumor progression and could become a new therapeutic target.

RESULTS

1) EMMPRIN is overexpressed in CLL cells of progressive cases compared with indolent counterpart

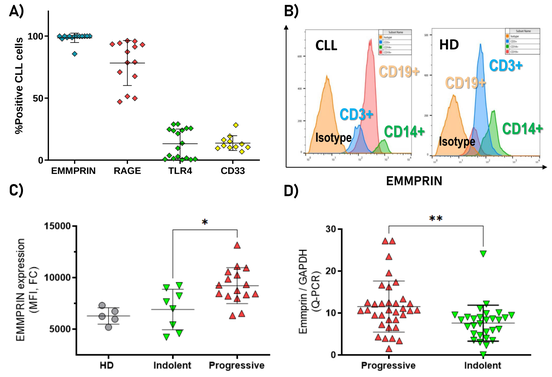

Figure 1: A) Evaluation of S100A9 receptors: EMMPRIN, RAGE, TLR4 and CD33 receptors in CD19+CD5+ cells. **B)** Representative expression of EMMPRIN in immune cells populations, including B cells, T cells, and monocytes from CLL patients and healthy donors (HD). **C)** Emmprin expression in CD19+CD5+ cells of progressive, indolent patients and HD (patients were classified as described in Prieto et al., Blood, 2017) was analyzed by flow cytometry (FC) ($p \leq 0.05$, t-test). **D)** EMMPRIN mRNA expression levels were quantified by quantitative PCR (qPCR) in progressive and indolent CLL patients ($p \leq 0.05$, t-test).

RESULTS

2) EMMPRIN Receptor is N-Glycosylated in CLL Cells, and high expression associated with unmutated IgHV Profile and early treatment

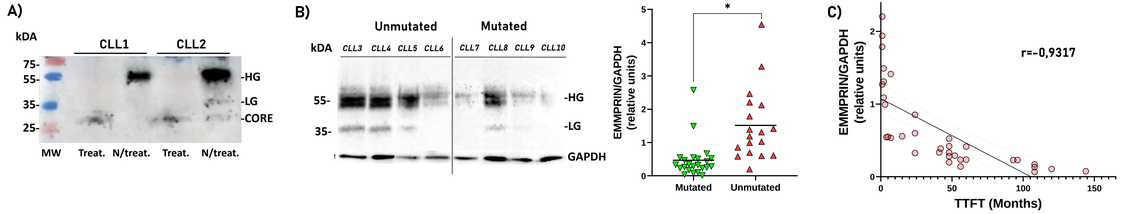

Figure 2: A) CLL protein lysates were treated with N-glycosidase F (PNGase F) for 3 hs and EMMPRIN was evaluated by Western Blot (WB). **B)** Representative WB of EMMPRIN in progressive and indolent CLL. The two glycosylated forms of EMMPRIN High (HG) and Low (LG) are visible in the blot, and the graph shows the relative protein expression normalized to GAPDH, which serves as a loading control ($p \leq 0.05$, t-test). **C)** Negative correlation between EMMPRIN expression and time to first treatment (TTFT) (n=35, Spearman r= -0,9317, p= <0,0001)

3) S100A9 promotes expression and phosphorylation of proteins associated with cell survival and tumor progression in CLL

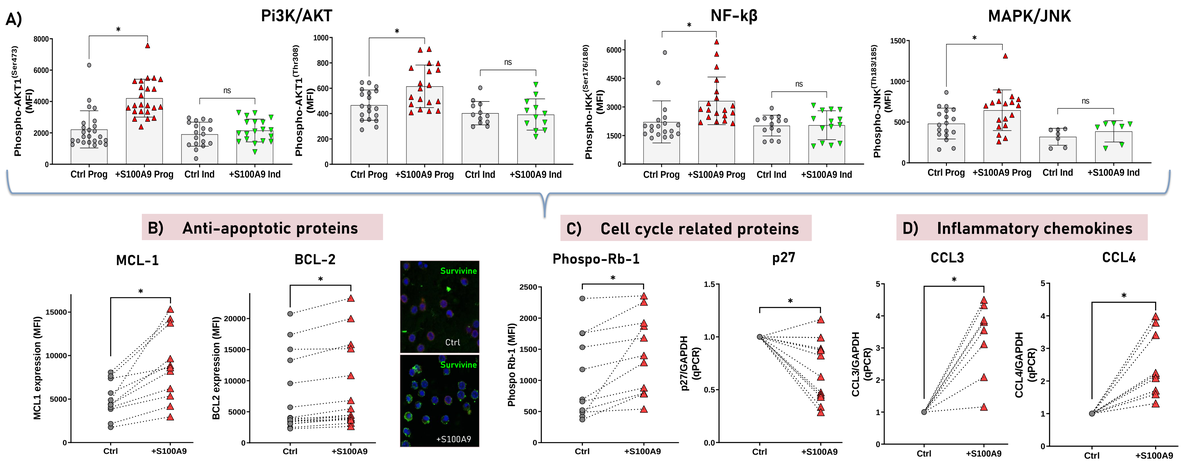

Figure 3: CLL cells were incubated with human recombinant S100A9 for 72 hours, and different proteins were evaluated by FC and qPCR. **A)** Phosphorylation levels of AKT1(Ser473 and Thr308), IKK(Ser176/180) and JNK(Thr183/185) were evaluated in CD19+CD5+ cells compared to unstimulated cells (Ctrl) by FC ($p \leq 0.05$, paired t-test) **B)** Anti-apoptotic proteins MCL-1 and BCL-2 were evaluated in CD19+CD5+ cells by FC. Survivine was evaluated by confocal microscopy. **C)** Cell-cycle related proteins phospho-Retinoblastome-1 (pRB) was evaluated in CD19+CD5+ cells by FC, and p27 mRNA expression was evaluated by qPCR ($p \leq 0.05$, t-test). **D)** Inflammatory chemokines CCL3 and CCL4 were evaluated by qPCR ($p \leq 0.05$, t-test).

4) Inhibition of S100A9/EMMPRIN axis decrease phosphorylation of Pi3k/AKT, NF-kβ and MAPK/JNK

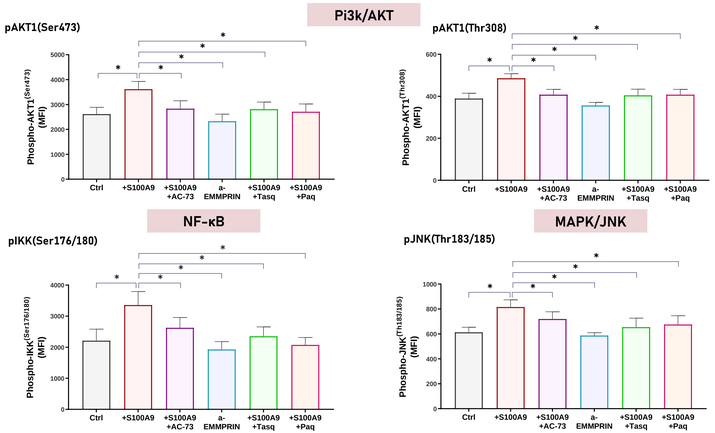

Figure 4: CLL cells were stimulated with S100A9 and treated with S100A9 inhibitors (Tasquinimod 10μM or Paquinimod 10μM), EMMPRIN inhibitor (AC-73 1μM) or α-EMMPRIN (blocking antibody 10μg/mL) for 72 hours. **A)** Phospho-AKT1 Ser473 (n=15) and Thr308 (n=8), **B)** Phospo-IKK Ser176/180 (n=15) and **C)** Phospo-JNK Thr183/185 (n=8) were assessed by FC in CD19+CD5+ cells. Cells without S100A9 stimulation were used as controls. Statistical significance was observed ($p \leq 0.05$, t-test).

CONCLUSIONS

- ❖ **EMMPRIN is significantly overexpressed in B-CLL cells of progressive patients and associated with TTFT.**
- ❖ **Stimulation with S100A9 increases phosphorylation of AKT, IKK, and JNK in B-cells of progressive patients, suggesting the activation of these pathways.**
- ❖ **Inhibition of the S100A9/EMMPRIN axis suggests a decrease in these pathways, commonly associated with tumor progression and cell survival in CLL cells.**

Inhibition of S100A9/EMMPRIN axis could be a promising therapeutic strategy for the treatment of CLL

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