(PF570) CEBPB COUPLES B-CELL RECEPTOR SIGNALING TO CLL CELL PROLIFERATION AND SURVIVAL

Topic: 05. Chronic lymphocytic leukemia and related disorders - Biology & translational research

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Background

BCR signaling in CLL microenvironment is one of the key drivers of proliferation and survival. However, it is not fully known how BCR activation triggers massive gene expression changes resulting in CLL cells' proliferation and survival.

Aims

To identify a key transcription factor (TF) involved in signaling pathways essential for the survival and/or proliferation of CLL cells in the microenvironment and test potential of its therapeutic inhibition.

Methods

ChIP-seq and RNA-seq analysis from BCR-stimulated primary CLL cells (bead-bound anti-IgM, 250 ng/10e6 cells). Gene editing and pharmacological inhibition of CEBPB performed in proliferating CLL cells using Cas9-gRNA ribonucleoprotein complex and antagonistic peptide lucicebtide, respectively.

Results

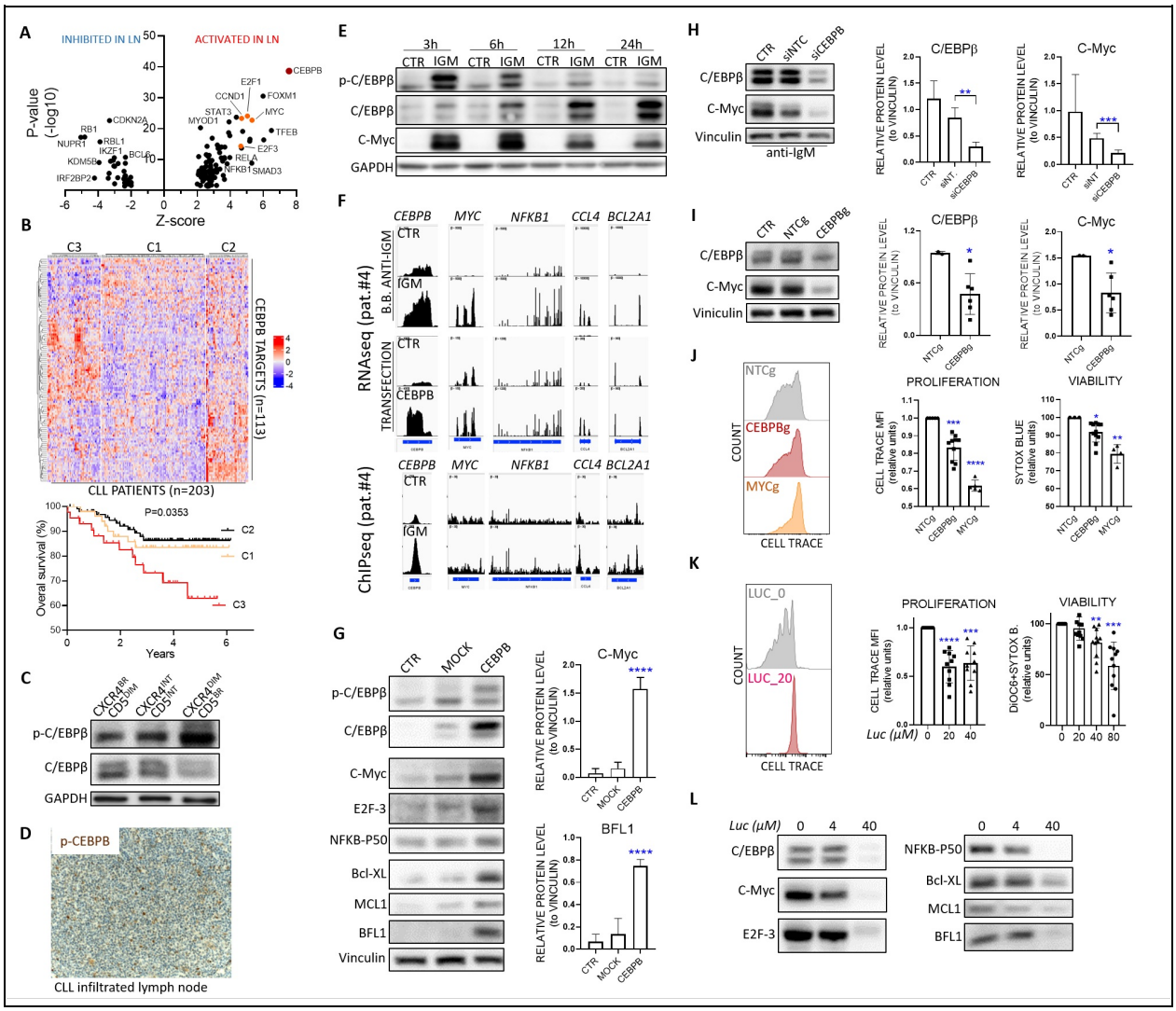
Re-analysis of transcriptomic data from CLL samples isolated from peripheral blood (PB) and lymph nodes (LNs) (n=29 pairs, Sun et al., 2023) revealed CEBPB as the most significant transcriptional regulator of genes activated in the LN microenvironment (IPA, **Fig. A**). Notably, the increased expression of the mRNAs regulated by CEBPB associated with worse prognosis in CLL (**Fig. B**). The analysis of CXCR/CD5 intraclonal CLL subpopulations (n=10 pairs) showed enrichment of transcriptionally active p-CEBPB (Thr235) in proliferative $CXCR4^{dim}CD5^{bright}$ cells (n=8; **Fig. C**), and p-CEBPB protein levels were clearly detected in CLL pseudofollicles in LNs (IHC, **Fig. D**). We tested a panel of 7 microenvironmental factors (anti-IgM, CD40L, IL4, IL6, IFNa/g, CpG-ODN), revealing that BCR activation (bead-bound anti-IgM) was the strongest inducer of CEBPB phosphorylation and expression (**Fig. E**). CEBPB overexpression in primary CLL cells (via transfection of plasmid) induced expression of genes activated in LN-resident CLL cells and involved in CLL proliferation and survival (MYC, NFKB-P50, BCL2 family; at both mRNA and protein levels; **Fig. F top**, **G**). ChIPseq for CEBPB revealed its direct binding to the regulatory regions of such genes, especially after BCR activation (6 hrs, **Fig. F bottom**). Notably, *CEBPB* silencing (siRNA in primary CLL, n=5) demonstrated that BCR-induced C-Myc expression is dependent on CEBPB (**Fig. H**), suggesting that CEBPB directly regulates this key cell proliferation driver. Furthermore, we observed that *CEBPB* knockdown (transfection of Cas9-gRNA ribonucleoprotein complex) in proliferating primary CLL cells (after pro-proliferative stimulation with anti-IgM, CD40L, IL4, IL21, CpG) leads to a decrease in C-Myc (n=6), impaired proliferation (n=10), and reduced viability (n=11; **Fig. I, J**). Finally, we evaluated blocking CEBPB function with the antagonist peptide lucicebtide, which is clinically tested in glioblastoma (trial NCT04478279). Lucicebtide strongly reduced CLL cell proliferation (n=10) and viability in dose dependent manner (n=11; **Fig. K**) and decreased C-Myc, NFKB-p50, and BCL2 family proteins (n=3 for each;**Fig. L**).

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Summary/Conclusion

We identify CEBPB as a transcriptional factor activated by BCR signaling in CLL cells. CEBPB is a key driver of gene expression differences in lymph node-resident CLL cells and is involved in their proliferation and survival by inducing C-Myc, BCL2 family, and NFkB activity. CEBPB can be inhibited by antagonist peptide lucicebtide in therapeutically-relevant concentrations.

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