(PF569) STAT1 SIGNALING DRIVES MICROENVIRONMENT-MEDIATED VENETOCLAX RESISTANCE AND PROLIFERATION IN CLL

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

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Background

Venetoclax-based therapy has substantially improved CLL outcomes; however, resistance occurs and is mainly driven by the tumor microenvironment (TME) in lymph nodes (LNs) (Thijssen et al. 2015, 2022). The key signaling pathways sustaining CLL proliferation and venetoclax resistance remain incompletely defined.

Aims

To identify TME-derived signaling pathways driving venetoclax resistance and CLL proliferation, and to evaluate JAK-STAT inhibition as a strategy to restore venetoclax sensitivity.

Methods

Primary CLL cells (n=32) were stimulated with TME factors or stromal co-culture (model by Hoferkova et al. 2024), and in presence of venetoclax ± JAK inhibitors. CRISPR was performed in primary CLL cells using Cas9-gRNA transfection.

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Results

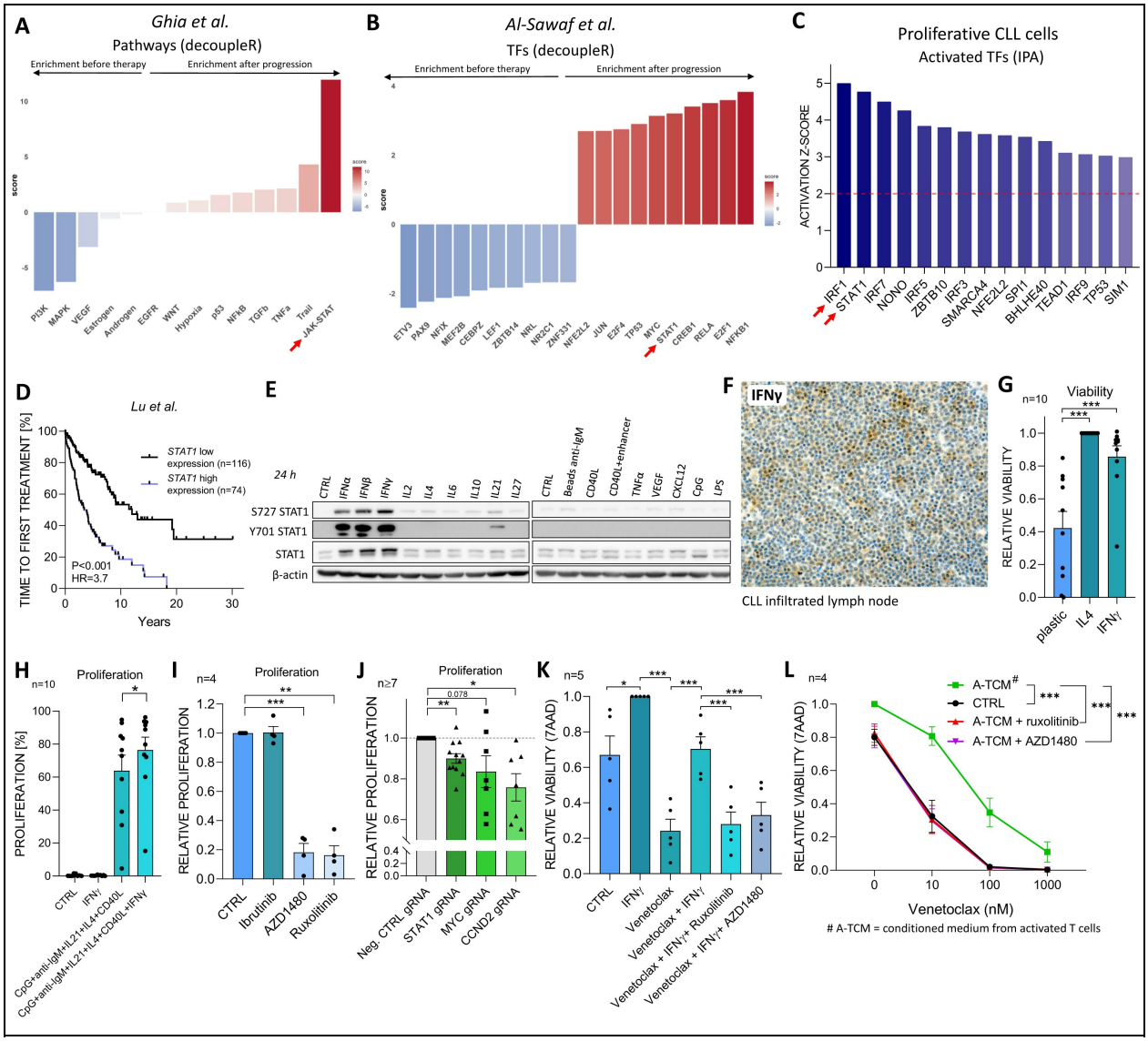
Re-analysis of transcriptomic data from CLL samples obtained at relapse post venetoclax (n=7 pairs, Ghia et al. 2022; n=29 pairs, Al-Sawaf et al. 2023) revealed strong enrichment of JAK-STAT and IFNγ signaling (IPA, GSEA) with STAT1 among the top predicted transcriptional regulators in 2 independent datasets (**Fig.A,B**). To determine whether this pathway also contributes to LN-driven proliferation/survival, we performed transcriptomic analysis of CXCR4/CD5 intraclonal CLL subpopulations (n=10 pairs). JAK-STAT signaling was markedly activated in proliferative $CXCR4^{dim}CD5^{brigh}$ cells, with STAT1 and its target IRF1 showing the highest regulatory activity (**Fig.C**). Increased STAT1 activity was independently confirmed in LN-resident CLL cells (n=29 pairs, Sun et al. 2023). Clinically, higher *STAT1* expression was associated with shorter time-to-first-treatment and OS across 2 independent CLL cohorts (n=191, n=106, **Fig.D**). Functional screening of 16 TME stimuli identified IFNγ and IL21 as the main inducers of STAT1 phosphorylation in primary CLL cells (**Fig.E**). CLL-derived T cells (CD3/28-activated) produced both IFNγ (312 ng/ml) and IL21 (1 ng/ml), induced STAT1 activation, and promoted CLL cell proliferation. Despite the immunologically "cold" nature of CLL LNs, immunohistochemistry confirmed IFNγ cytokine expression in CLL LNs (n=8; **Fig.F**). IFNγ stimulation significantly enhanced CLL cell viability (**Fig.G**) and cooperated with other TME stimuli to boost proliferation (**Fig.H**), while IL21 strongly promoted CLL proliferation in the co-culture model (10% vs. 49%, n=16, $P<0.05$). Notably, JAK inhibition (ruxolitinib, AZD1480; **Fig.I**) or *STAT1* knockout in primary CLL cells (CRISPR; **Fig.J**) impaired TME-driven proliferation. ChIP-seq for STAT1 revealed regulation of multiple pro-survival and pro-proliferative genes in CLL cells following IFNγ or IL21 treatment or co-culture. IFNγ stimulation effectively rescued CLL cells from venetoclax-induced apoptosis (**Fig.K**), whereas IL21 had no significant effect. This was accompanied by upregulation of the anti-apoptotic MCL1 and BCLxL by IFNγ. Importantly, JAK inhibition (ruxolitinib or AZD1480) restored venetoclax sensitivity across multiple *in vitro* models, including IFNγ stimulation, T-cell-conditioned media, and stromal co-culture (**Fig.K,L**).

Summary/Conclusion

We identify STAT1 signaling as a key TME–driven pathway promoting CLL cell proliferation, survival, and TME-mediated resistance to venetoclax. JAK inhibition consistently restored venetoclax sensitivity across TME models, providing a strong rationale for combining JAK inhibitors with venetoclax.

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