IFNγ/IL21-JAK-STAT1 Signaling Axis Drives T-Cell–Mediated CLL Proliferation and Venetoclax Resistance

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Introduction

The lymph node microenvironment is essential for CLL cell survival and proliferation. Various factors such as CD40L, IL21, or BCR signaling have been implicated in the induction of CLL cell proliferation, however, no single factor is sufficient to stimulate proliferation on its own. Despite extensive research, the identification of key proliferation drivers *in vivo* remains incompletely understood. It has been shown that multiple T cell derived factors can contribute to activation of key transcription factors such as MYC, NF-κB, or E2F (Herishanu et al., 2011), however, this is mostly based on pre-existing knowledge from biology of normal germinal center B cells. The goal of our study was to identify key signals from the CLL microenvironment that contribute to proliferation of leukemic cells.

Methods

Transcriptomic profiling was performed on sorted CXCR4^{dim}CD5^{bright} (proliferative) and CXCR4^{bright}CD5^{dim} (resting) CLL subpopulations (**Fig. 1A**). To model T–CLL cell interactions, we used co-culture with engineered HS5 cells expressing CD40L, IL4, and IL21 (HS5$^{CD40L\text{-}IL4\text{-}IL21}$, Hoferkova et al., 2024), stimulation with a proliferative cocktail (CD40L+IL4+IL21+α-IgM+CpG), and stimulation with conditioned medium from CD3/28-activated T cells (CLL patient derived, A-TCM). CRISPR/Cas9 gene editing was used to downregulate STAT1 in primary CLL cells. *miR-150* levels were modulated by lentiviral overexpression in HG-3 cells or by *miR-150* mimic transfection in primary CLL cells. JAK inhibitors (ruxolitinib, AZD1480, both 1 μM) were tested in combination with venetoclax to assess effects on viability and drug resistance. Statistical significance in figures is represented as follows: *p < 0.05, **p < 0.01, ***p < 0.001.

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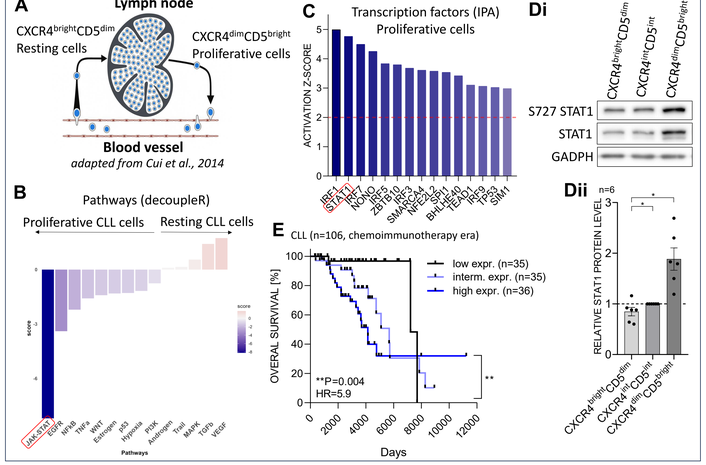
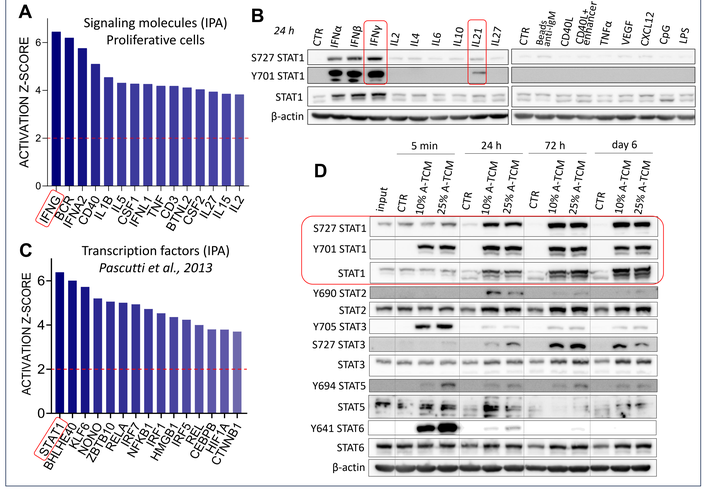
1. JAK/STAT1 signaling is activated in proliferative CLL cells

RNA profiling revealed JAK–STAT as the most enriched pathway in proliferative CXCR4^{dim}CD5^{bright} cells (**Fig. 1B**), with STAT1 as the top activated transcription factor (**Fig. 1C**). Re-analysis of LN vs. PB dataset (Sun et al., 2023) confirmed STAT1 activation (data not shown), and immunoblotting showed elevated pSTAT1 and total STAT1 level in proliferative CLL cells (**Fig. 1Di,ii**). High STAT1 expression correlated with shorter overall survival, supporting its association with aggressive disease (**Fig. 1E**).



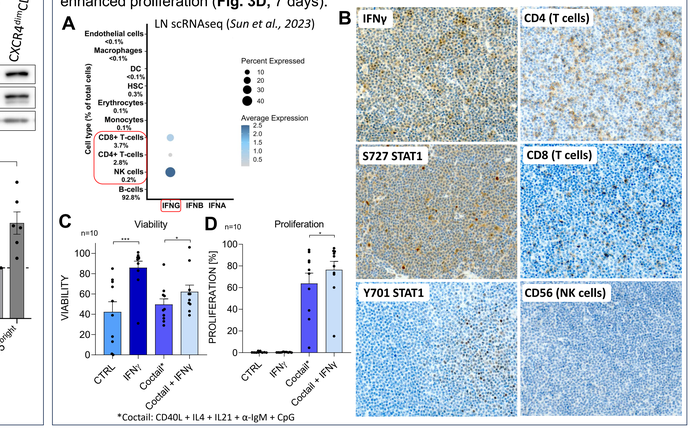
2. T cells activate STAT1 in CLL cells via IFNγ and IL21

Upstream regulator analysis predicted IFNγ as a top driver of gene expression changes in proliferative CLL cells (**Fig. 2A**). Screening of 16 microenvironmental stimuli identified T cell-derived factors IFNγ and IL21 as strong inducers of STAT1 phosphorylation at Y701 and S727 in primary CLL cells (**Fig. 2B,** 24 h). Co-cultivation with T cells (Pascutti et al., 2013 data) confirmed STAT1 as top activated transcription factor (**Fig. 2C**). Experimentally, conditioned medium from activated T cells produced sustained STAT1 phosphorylation, highlighting T cell cytokines as dominant STAT1 stimuli (**Fig. 2D**).



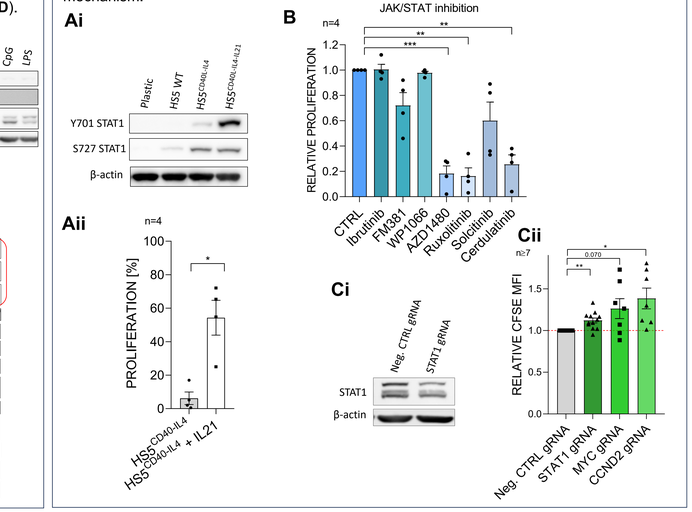
3. IFNγ-STAT1 axis promotes proliferation and survival of CLL cells

Re-analysis of scRNAseq data (Sun et al., 2023) identified high expression of IFNG gene in LNs predominantly by T and NK cells, with negligible IFNA/IFNB expression (**Fig. 3A**). Immunohistochemistry confirmed IFNγ presence together with pSTAT1 in LN-resident CLL cells (**Fig. 3B**). Moreover, we confirmed the presence of CD4+ and CD8+ T cells in LN, in contrast to NK cells which were hardly detectable (**Fig. 3B**). IFNγ stimulation significantly increased CLL cell viability (**Fig. 3C,** 7 days), and when combined with other cytokines, enhanced proliferation (**Fig. 3D,** 7 days).



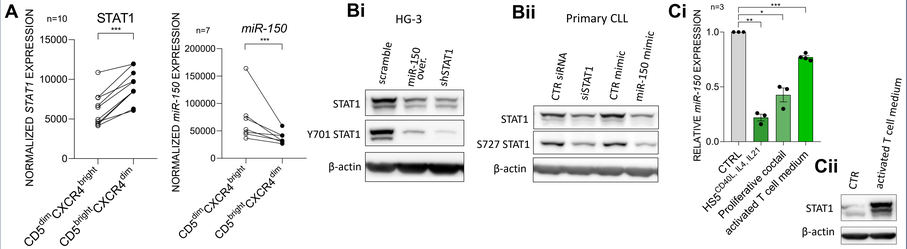
4. IL21-STAT1 axis promotes proliferation of CLL cells

The expression of IL21 in CLL niches by T cells has been documented previously (Pascutti et al., 2013). IL21 triggered STAT1 phosphorylation (**Fig. 4Ai**, 24 h) and robust proliferation of primary CLL cells in HS5$^{CD40L\text{–}IL4}$ co-culture (**Fig. 4Aii,** 7 days). This effect was blocked by JAK inhibitors (ruxolitinib, AZD1480 and cerdulatinib, **Fig. 4B,** 7 days) or by STAT1 downregulation using CRISPR-Cas9 (**Fig. 4Ci,ii,** 8 days), demonstrating a STAT1-dependent mechanism.



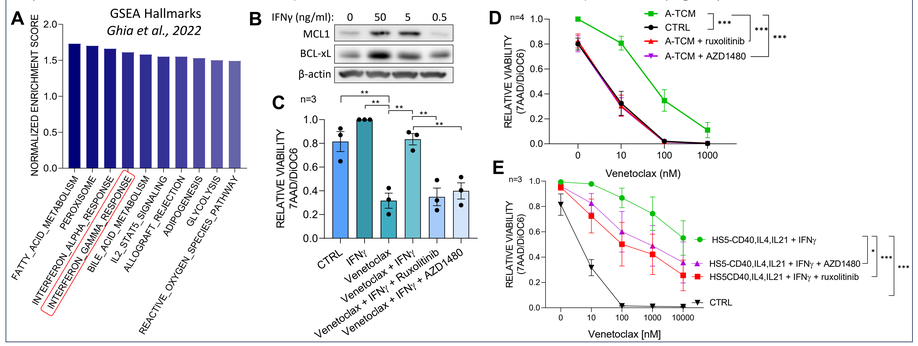
5. *miR-150* regulates STAT1 in response to microenvironmental stimuli

In addition to STAT1 phosphorylation being triggered by IFNγ and IL21, we noted that its levels are controlled post-transcriptionally by *miR-150*. *miR-150* was downregulated in proliferative CLL cells and inversely correlated with STAT1 expression (**Fig. 5A**). *miR-150* overexpression reduced STAT1 levels in CLL-derived HG-3 cells and primary CLL cells (**Fig. 5Bi,ii**). Proliferative stimuli from the microenvironment, including stromal co-culture (7 days), stimulation with proliferative cocktail (5 days) and T cell conditioned media (25%, 6 days), repressed *miR-150* expression, contributing to STAT1 upregulation (**Fig. 5Ci,ii**).



6. IFNγ-induced venetoclax resistance is reversed by JAK/STAT inhibition

Next, we hypothesized that the IFNγ/IL21-JAK-STAT1 axis might promote venetoclax resistance driven by T-CLL cell interactions. Re-analysis of transcriptomes from patient samples who had progressed on venetoclax therapy (Ghia et al., 2022) revealed significant IFNγ-pathway activation (**Fig. 6A**), while IL21 signaling was not activated. In line with this, IFNγ induced MCL1 and BCL-xL upregulation (**Fig. 6B**). IFNγ treatment completely rescued CLL cell apoptosis induced by 10 nM venetoclax (**Fig. 6C**). JAK inhibition with ruxolitinib or AZD1480 reversed venetoclax resistance induced by IFNγ (**Fig. 6C**), or conditioned media from activated T cells (**Fig. 6D**). Moreover, JAK inhibition reduced the protective effect of HS5$^{CD40L\text{–}IL4\text{–}IL21}$ with IFNγ co-culture (**Fig. 6E**).



Conclusion

- STAT1 is the key transcription factor activated by T cell–produced IFNγ and IL21
- IFNγ is present in CLL lymph nodes, produced mainly by CD8+ and CD4+ T cells
- STAT1 promotes CLL cell proliferation and survival
- STAT1 expression is repressed by *miR-150*
- IFNγ induces venetoclax resistance, which is reversed by JAK/STAT inhibition