LYN kinase programs stromal fibroblasts to facilitate leukemic survival via regulation of c-JUN and THBS1

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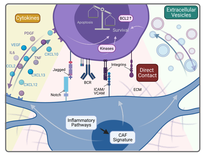
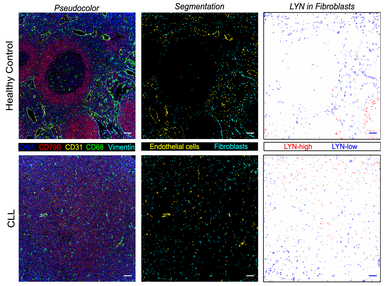
Background

- CLL is dependent on the supportive tumor microenvironment (TME)
- Understanding the TME and support mechanisms will help to optimize therapy and overcome resistance
- Fibroblasts/mesenchymal stromal cells are an important cell type in the CLL TME, supporting CLL in various ways

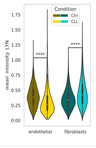


LYN kinase is overexpressed in stromal cells of the CLL TME



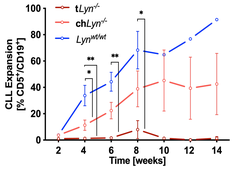
Representative images of Hyperion Imaging Mass Cytometry (IMC) of a healthy control lymph node (HC-LN) (*top*) and a CLL-LN (*bottom*) depicting marker expression (*left*), segmentation of Vimentin+ fibroblasts (*middle*) and pseudocolor LYN intensity in segmented fibroblasts.

- IMC enables detection of fibroblasts in the CLL lymph node microenvironment
- **CLL associated LN-fibroblasts have a significantly increased LYN expression compared to healthy control LN-fibroblasts**



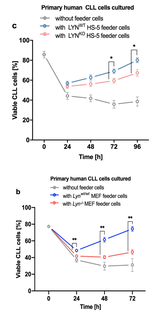
Quantification of IMC analysis reveals significantly increased LYN expression in CLL LN fibroblasts compared to healthy control lymph nodes. (Healthy controls: n = 9 HC-LN; CLL: n = 12 CLL-LN)

Stromal LYN kinase promotes CLL *in vivo* and *in vitro*



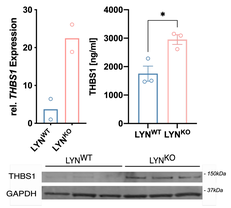
Reduced TCL1+ leukemia progression in mice, lacking LYN kinase specifically in the non-hematopoietic TME (chLyn-/-). Mice were lethally irradiated and bone-marrow transplanted to generate specific LYN-deficiency in the non hematopoietic TME (chLyn-/-) or complete TME (tLyn-/-). TCL1+ leukemic cells were transplanted and PB was analyzed.

- **LYN expression in fibroblasts promotes their leukemic support**
- Lyn deficiency in the non-hematopoietic microenvironment reduces leukemia progression in a TCL1-mouse model *in vivo*
- LYN deficient fibroblasts have a reduced CLL supportive capacity in an *in vitro* coculture assay

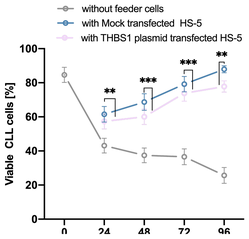


LYN deficient fibroblasts have reduced leukemia supportive capacity *in vitro*. LYN^{KO} HS-5 cells (top) and MEF from $Lyn^{-/-}$ mice (bottom) were cocultured with primary human CLL cells and viability was measured by flow cytometry.

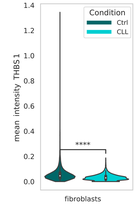
Increase in THBS1 expression restrains CLL survival



Increased THBS1 expression is validated in LYN deficient fibroblasts. qPCR (*upper left*) validates increased transcription and ELISA of the supernatant (*upper right*) and Immunoblotting of cell lysate (*lower*) validates increased protein expression.



THBS1 expression impairs CLL cell viability. HS-5 cells transfected to overexpress THBS1 show a reduced CLL supportive capacity over time in coculture with primary CLL samples.



Reduced THBS1 expressio in the CLL LN. IMC-Analysis of fibroblasts in the LN-microenvironment in CLL patients shows reduced THBS1 expression, associated with increased LYN expression in these cells.

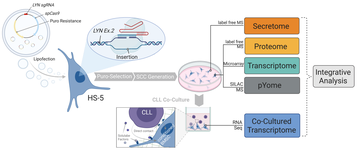
- THBS1 expression is transcriptionally decreased in LYN deficient fibroblasts
- **The extracellular matrix protein THBS1 actively reduces CLL viability by ligation to CD47 on leukemic cells.**
- The CLL LN-TME shows reduced levels of THBS1, which are associated with increased LYN expression and might promote leukemic development

SUMMARY

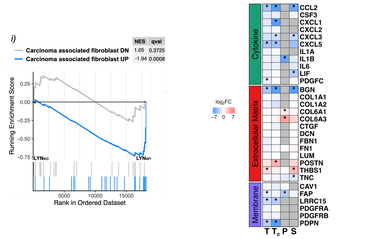
- **LYN kinase is active in fibroblasts of the CLL microenvironment and promotes a CAF-like repolarization affecting especially cytokine and matrix secretion**
- **Reduced c-Jun/AP1 activity upon LYN deficiency transcriptionally reprograms fibroblasts**
- **Reduced LYN/c-JUN axis translates into increased THBS1 expression, contributing to CD47 induced CLL cell apoptosis**

Multi-OMICs reveals fibroblast reprogramming and reduced CAF phenotype upon LYN deficiency

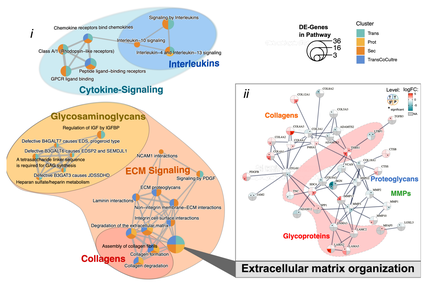
- HS-5 LYN^{WT} and LYN^{KO} cells were subjected to Multi-Omic analysis
- LYN deficient fibroblasts show a trascriptionally altered reprogramming
- **Many phenotypic changes were related to a modulated CAF polarization**
- Inflammatory cytokines and extracellular matrix secretion were altered most profoundly



LYN^{KO} was generated in HS-5 cells using CRISPR-Cas9 and cells were subjected to Multi-Omics profiling including Transcriptome (T), Proteome (P), Secretome (S), Phosphoproteome (Y) and Transcriptome after CLL contact (Tc). Results were analyzed integrativly.

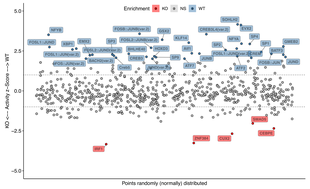


LYN deletion disturbs the CAF-like phenotype and polarization of stromal cells. GSEA analysis on transcriptomic data shows enrichment of an activated CAF geneset (as defined by by Mishra et al. (M18292 *blue* and M4577 *gray*) in LYN^{WT} HS-5 cells (*left*) and typical CAF markers are significantly deregulated in LYN^{KO} HS-5 cells in the different Multi-Omic levels (*right*)

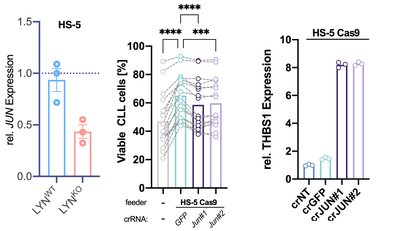


LYN deletion in stromal cells predominantly alters cytokine and matrix related pathways. (*i*) Enrichment map of the top enriched Reactome-pathways in the Multiomic Analysis shows clustering of Cytokine-Signaling and ECM-Signaling related pathway. Each node represents one Reactome-Term, the circle colors indicate "omics"-layers in which the pathway was deregulated (*ii*) STRING protein association network contributing to "ECM organization"

Transcriptional reprogramming by reduced c-Jun

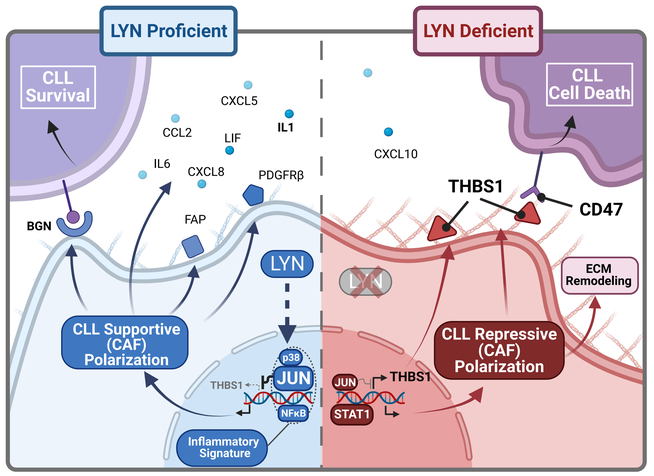


Epigenomics validats transcriptional reprogramming and shows reduced AP1 activity. Activity plot of transcription factor footprinting analysis in HS-5 Epigenome showing the z-transformed activity scores of the underlying motifs. Motifs with differential footprinting are highlighted in red (enriched in LYN^{KO}) or in blue (enriched in LYN^{WT})



LYN^{KO} HS-5 cells show reduced c-Jun expression and Jun-knockdown cells have reduced CLL feeder support and increased THBS1 expression. (*Left*) qPCR validates reduced c-Jun expression in LYN^{KO} HS-5 cells. Functional relevance of Jun was tested in Jun-knockdown HS-5 cells, stably expressing Cas9. Jun^{KO} cells show a reduced CLL supportive capacity in co-culture for 48h with primary CLL cells (*middle*). These Jun^{KO} cells show increased THBS1-mRNA expression in qPCR.

- Transcriptional reprogramming upon LYN deficiency in fibroblasts was corroborated by ATAC-Seq.
- **LYN^{KO} cells showed reduced activity in AP1 motifs and a reduced c-Jun expression was validated**
- Stromal c-Jun is functionally relevant for supporting CLL cell viability
- c-Jun regulates transcription of the ECM protein THBS1



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