LYN kinase and its regulator CSK as key players in CLL-supporting macrophages

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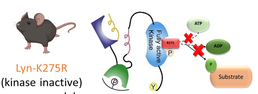
INTRODUCTION

- Chronic lymphocytic leukemia (CLL) cells are highly dependent on their surrounding cells and soluble factors - the tumor microenvironment (TME)
- Macrophages are key players in supporting CLL progression and survival
- Expression of B-cell receptor-associated tyrosine kinases like LYN and BTK in macrophages were shown to contribute actively to the formation of a leukemia-promoting niche

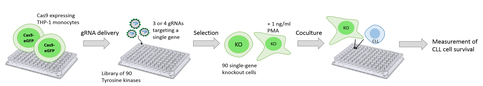
- **Hypothesis**: Tyrosine kinases in macrophages play an important role in the formation of a pro-leukemic environment leading to CLL cell survival

METHODS



Generation of a Lyn kinase inactive mouse model

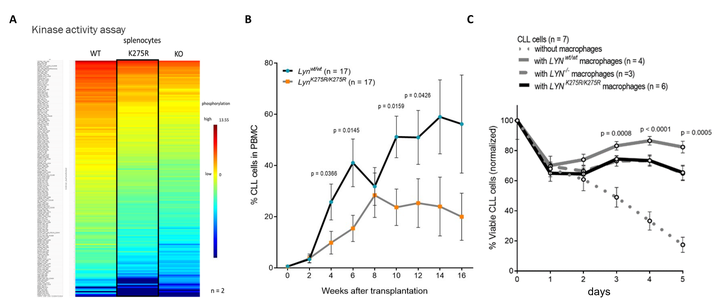
- A novel kinase inactive mutant mouse model was generated by CRISPR/Cas9-mediated homology-directed repair
- A targeted amino acid substitution (K275R) in the activation loop of the Lyn protein rendered the kinase enzymatically inactive



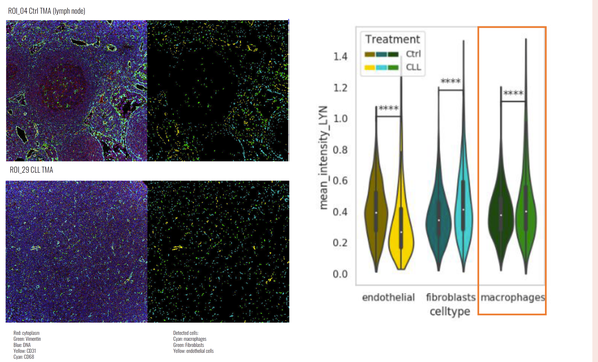
Arrayed CRISPR/Cas9 knockout screen of THP-1 cells

- THP-1 monocytes expressing the Cas9 nuclease were transduced with lentiviral particles containing 3 or 4 sgRNAs targeting one gene
- All known 90 tyrosine kinases were targeted
- Monocytes were differentiated with PMA into macrophages
- Macrophages and CLL patient cells (n=6) were cocultured together, and CLL cell viability was measured via flow cytometry

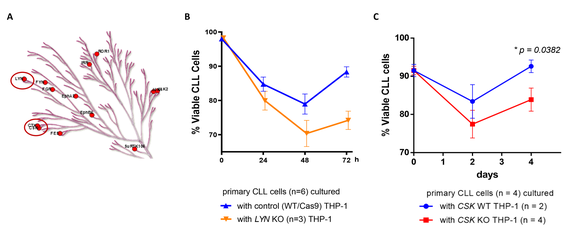
RESULTS



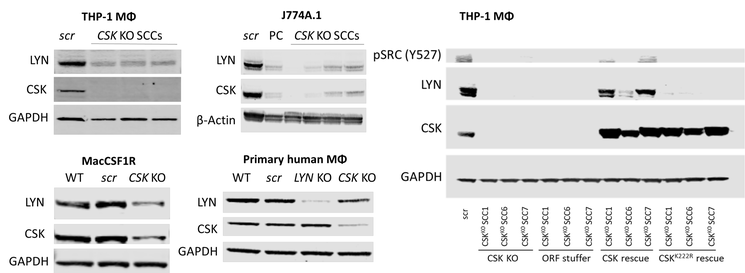
Lyn kinase inactive TME hinders CLL growth
The novel mouse model with a Lyn kinase inactive function shows a reduced phosphorylation pattern (A). After transplantation of *Eμ-TCL1* CLL cells into Lyn WT and Lyn K275R recipients, CLL development in the blood was significantly reduced in Lyn kinase inactive mice (B). Lyn kinase inactive bone marrow derived macrophages showed a reduced feeding capacity to primary CLL cells (C).



Increased LYN expression in CLL-associated macrophages
Tissue microarrays of healthy control and CLL lymph nodes were stained with metal-labeled antibodies for LYN expression and lineage markers and subjected to imaging mass cytometry. Macrophages in CLL lymph nodes exhibited elevated LYN protein levels in comparison to healthy controls.

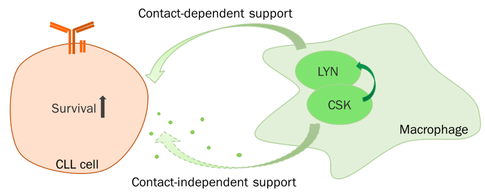


LYN and CSK deletion in THP-1 macrophages impairs CLL cell survival
The arrayed CRISPR/Cas9 knockout screen identified 13 tyrosine kinases, which deletion in THP-1 macrophages reduced CLL cell survival (A)
Cocultures of primary CLL cells with single sgRNA CRISPR/Cas9 knockouts of LYN (B) and CSK (C) in THP-1 cells validated the reduced feeding capacity.



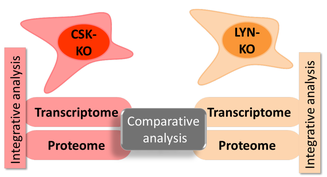
Deletion of CSK results in a reduction of LYN protein levels
Knockdown or knockout of CSK in the macrophage cell lines THP-1, J774A.1, MacCSF1R as well as in primary human macrophages led to a notable reduction of LYN protein levels.
Rescue of CSK in THP-1 cells also recovered LYN expression, but kinase inactive CSK (K222R) was not able to rescue LYN protein.

CONCLUSION



- LYN kinase activity in macrophages is essential for CLL-feeding capacity
- CRISPR/Cas9 screen in THP-1 cells identifies LYN and CSK as important for CLL cell survival
- Deletion of CSK reduces protein levels of LYN, dependent on the kinase activity of CSK
- First comparative analysis of the transcriptome of CSK and LYN knockout THP-1 macrophages shows shared and exclusive pathways

OUTLOOK



- The underlying mechanisms by which CSK and LYN in macrophages exert their prosurvival effects on CLL cells will be unraveled via multi-omics analyses
- The supportive effect of CSK, LYN and their kinase activity will be validated in additional cell lines

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abstract ID:1553624