I. Abstract

Chronic lymphocytic leukaemia (CLL) is the most common B-Cell malignancy in the western world. CLL cell survival is strictly dependent on a nurturing microenvironment, which influences the leukemic cells in a complex multi-directional cross-talk.

Besides its initially identified role as a central effector of the B-Cell receptor signalling pathway, LYN kinase was demonstrated to have multiple functions in cells of the haematopoietic and non-haematopoietic system. LYN was shown to be involved in numerous cellular processes such as proliferation, migration and adhesion, which renders this kinase a suitable target in cancer therapy. Our research group could show that LYN was essential for the formation of a microenvironment, enabling leukaemia progression in the CLL mouse model. In addition, murine Lyn-deficient macrophages showed strongly impaired feeding capacity towards CLL cells *in vitro,* rendering macrophages one very important cell type for CLL growth.

In this study, we addressed the relevance of LYN expression in a high-risk murine CLL model. We generated a murine model of TCL1-driven leukaemia with a B-Cell specific *Trp53* knockout and crossed them to global Lyn-deficient mice (*TCL1*$^{+/wt}$*; CD19Cre*$^{cre/wt}$*; p53*$^{fl/fl}$*; Lyn*$^{-/-}$*,* termed TCPL). TCPL animals have a significantly hindered CLL development compared to the respective TCP control cohort (*TCL1*$^{+/wt}$*; CD19Cre*$^{cre/wt}$*; p53*$^{fl/fl}$). TCPL mice have reduced CLL leukocyte count in the peripheral blood and show decreased infiltration of lymphoid tissues of moribund mice. TCPL mice are reminiscent of the phenotype of *TCL1*$^{+/wt}$*, Lyn*$^{-/-}$ animals, with a much more pronounced and severe phenotype, probably owing to the loss of *Trp53* in the B-Cell compartment. In addition, we transplanted CLL cells of the TCP animals in LYN$^{-/-}$ and WT animals and discovered a new transplantation phenotype distinctive from the *TCL1*$^{+}$ transplantation, with rapid infiltration to lymphoid organs prior to CLL cell circulation into the bloodstream, mimicking a (transformed) lymphoma model.

To provide further insights into LYN-associated underlying molecular mechanism in macrophages in a fully human co-culture system, we established a CLL-macrophages co-culture system with Lyn-wildtype versus Lyn-deficient THP1 macrophages as feeder cell lines. We characterized the role of LYN in this cell line to study the interaction with CLL cells regarding their nursing capacity by applying comprehensive

multi-omics analysis. Our findings revealed that the Lyn-dependent altered downstream signalling led to increased cellular adhesion of $LYN^{-/-}$ THP1 macrophages, whereas other major cellular processes are massively down-regulated.

In addition, we performed multiplex Immunohistochemistry of human CLL lymph node tissue showing that the LYN kinase in $CD68^{+}$ macrophages is significantly overexpressed compared to healthy control tissues, underlining the importance of LYN in CLL-associated macrophages.

Collectively, our results demonstrate that LYN expression is also important for the progression of high-risk Tp53-deficient CLL *in vivo*, and provide further insights into the mechanistic role of LYN in macrophages of the human CLL tumour microenvironment.