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Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

MIR-146B RESTORATION IN T-LARGE GRANULAR LYMPHOCYTE LEUKEMIA: MOLECULAR INSIGHTS FOR A NEW THERAPEUTIC STRATEGY AGAINST NEUTROPENIA DEVELOPMENT

A. Teramo[1|2], E. Rampazzo[1|2], E. Roncaglia[3|4], B. Mariotti[5], G. Calabretto[1|2], E. Buson[1|2], E. Gaffo[6], S. Orsi[1], R. Crivellari[1|2], G. Pertile[1|2], V. Trimarco[1], M. Facco[1], F. Bazzoni[4], L. Trentin[1], G. Semenzato[2], §S. Bortoluzzi[4], R. Zambello[1|2]

§: Co-last author.

[1]Department of Medicine, Hematology Unit, University of Padova; [2]Veneto Institute of Molecular Medicine - VIMM; [3]Department of Biology, University of Padova; [4]Computational Genomics Group, Department of Surgery, Oncology and Gastroenterology, University of Padova; [5]Department of Medicine, Division of General Pathology, University of Verona; [6]Department of Molecular Medicine, University of Padova.

Introduction: In T-Large Granular Lymphocyte Leukemia (T-LGLL), characterized by the clonal expansion of cytotoxic LGLs, neutropenia is the main clinical manifestation. Current immunosuppressive therapies have limited efficacy and high toxicity, highlighting the need for targeted approaches. Neutropenia development is reported to be due to high Fas Ligand (FasL) production by LGLs, which triggers neutrophil cell death. We recently observed that the downregulation of miR-146b can be responsible for the high expression of FasL, through STAT3-miR-146b-ELAVL1-FasL axis.

This project aimed to investigate the molecular consequences of miR-146b restoration in LGLs from both patients and healthy donors (HD), as the first step to proceed toward developing a potential RNA-based therapy for T-LGLL-related neutropenia.

Methods.

LGLs were isolated from 6 patients, while CD8+ cytotoxic T lymphocytes (CTLs) were purified from 6 HD. Cells were transfected with either a miR-146b mimic or a negative control (scramble). After 24 hours (h), cell viability and transfection efficiency were assessed, and total RNA was extracted to perform RNA sequencing. Gene expression was quantified using CircComPara. Differentially expressed gene (DEG) analysis was conducted using DESeq2 v1.46.0 and gene set enrichment analysis using clusterProfiler v4.14.6 and enrichplot v1.28.0.

RESULTS

MiR-146b uptake post-transfection was confirmed with a cell viability >60%. According to transcriptomic data, the changes between scramble and miRNA-transfected conditions are marked in patient LGLs (2,404 DEGs) and mild in HD (154 DEGs).

Our data confirmed that *ELAVL1* and *FasL* are over-expressed in T-LGLL patients compared to HD, and demonstrated that miR-146b restoration was able to successfully downregulate *ELAVL1* expression ($p<0.01$), leading to *FasL* reduction ($p<0.05$), thus reverting the pathogenic axis associated with neutropenia.

In addition, miR-146b is found to exert the desired effect not only on *ELAVL1*, but also on several other genes relevant to T-LGLL pathogenesis. Among these, *CCL5*, a chemokine implicated in LGL proliferation, and *SBNO2*, required in STAT3-dependent hematopoietic malignancies, were significantly reduced in miR-146b-transfected LGLs ($p<0.01$).

Additional analyses disclosed that, upon miR-146b restoration, many signaling pathways were enriched in genes downregulated in leukemic LGLs, whereas no pathway perturbation is revealed in HD. Finally, the expression of nearly half of the genes altered in the disease was completely restored or positively affected by the miR-146b restoration, while only a small proportion of genes not altered in the disease were affected, mostly mildly, by the treatment.

Conclusions

Our results support miR-146b restoration as a promising therapeutic strategy, selectively affecting leukemic cells with negligible effect on healthy CTLs, able to correct key targets implicated in T-LGLL-associated neutropenia and in LGL pathogenesis.

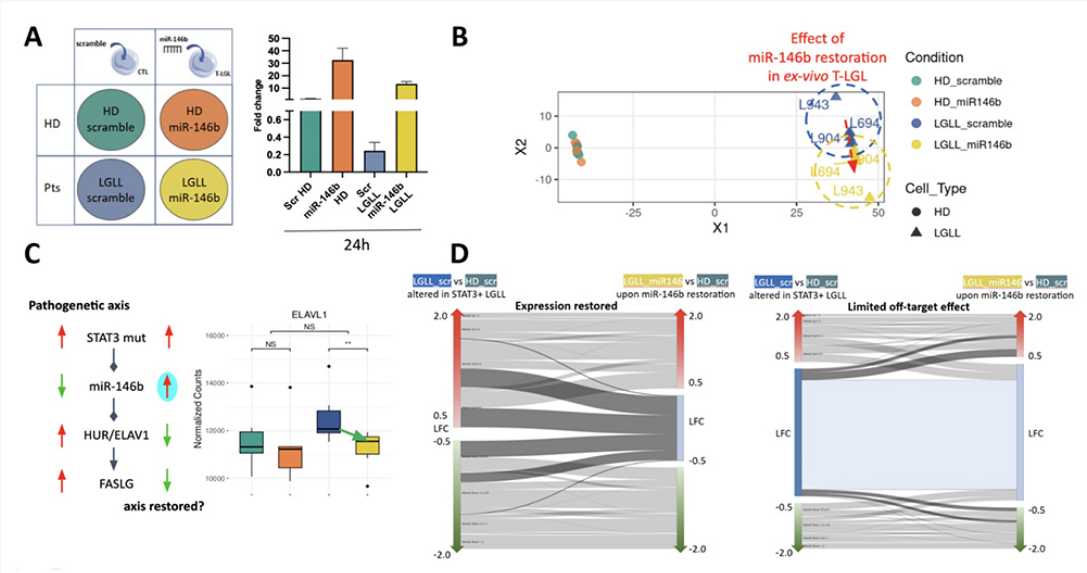

Figure 1. Panel A: Transfection with scramble control or miR-146b was performed in cytotoxic T lymphocytes (CTLs) from six healthy donors (HD) and in leukemic T-LGLs from six T-LGLL patients (Pts). The histogram shows miR-146b uptake in both culture conditions 24 h post-transfection. **Panel B**: Principal component analysis (PCA) of transcriptome data reveals a more pronounced effect of miR-146b restoration in T-LGLs than in CTLs from HD. **Panel C**: The pathogenic STAT3–miR-146b–ELAVL1–FasL axis, associated with neutropenia in T-LGLL, is normalized by miR-146b transfection. ELAVL1 (also called HUR) expression decreases to levels comparable to HD (**$p < 0.01$; NS, not significant), leading to reduced FasL expression. **Panel D**: The graph on the left shows that miR-146b restoration rescues the expression (LFC, Log fold change) of hundreds of genes dysregulated in STAT3-mutated T-LGLL to levels similar to those observed in HD. The one on the right shows that among genes not affected by the disease, only a small subset shows mild changes upon treatment.