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Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

STAT3-MEDIATED CIRCULAR RNAS DYSREGULATION IN NEUTROPENIC T-LARGE GRANULAR LYMPHOCYTE LEUKEMIA PATIENTS

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Introduction: T-Large Granular Lymphocyte Leukemia (T-LGLL) is a rare chronic lymphoproliferative disorder with unclear pathogenesis. Clonal expansion is driven by pro-inflammatory cytokines that induce JAK/STAT stimulation. Activating *STAT* mutations have been identified, with *STAT3* lesions being typical of CD8+ T-LGLL and distinguishing symptomatic cases, mainly for neutropenia, from indolent ones. The discovery of the STAT3-miR-146b-FasL axis in *STAT3*-mutated CD8+ cases has highlighted the role of non-coding RNAs (ncRNAs). This study aims to clarify the role of circular RNAs (circRNAs), a class of ncRNAs, whose involvement in T-LGLL remains unexplored.

Methods: We considered RNA-sequencing (RNA-seq) data (GEO GSE228868) of 20 T-LGLL cases and 5 healthy donors (HD). CircRNA identification and analysis was carried out using the CircCompara2/edgeR pipeline. Candidate circRNAs were validated in an independent cohort (n=26) through Sanger sequencing and RT-qPCR. Patient cells were treated with IL-6 (20 ng/µL) or the STAT3 inhibitor STATTIC (ST) (3.5 µM). After 24 hours cell viability, STAT3 activation, and circRNA expression were assessed via Annexin V staining, western blot, and RT-qPCR, respectively.

RESULTS

A total of 5,948 circRNAs ($p\text{-}adj<0.05$) were detected through RNA-seq. Principal component analysis revealed distinct circRNA expression patterns between T-LGLL patients and HD, with 358 circRNAs differentially expressed in neutropenic *STAT3*-mutated patients compared to asymptomatic ones. The altered expression of 11 circRNAs was validated in an independent cohort. Of these, 5 were specifically altered in *STAT3*-mutated CD8+ patients: 4 were up-regulated, while circZC3H12B was down-regulated. Among upregulated circRNAs, circPVT1 and circZBTB46 were previously associated with oncogenic roles in different malignancies, whereas circZC3H12B, previously known as circX, was found highly expressed in normal lymphocytes and downexpressed in Acute Lymphoblastic Leukemia. To assess the potential link between STAT3 activation and circRNA dysregulation, cells from asymptomatic patients were stimulated *ex vivo* with IL-6 which is known to trigger STAT3. Protein activation, confirmed by western blot ($p<0.01$), modulated the 5 circRNAs to levels comparable to symptomatic cases. Consistently, experiments on cells from symptomatic patients with STAT3 hyperactivating variants treated with ST, an inhibitor of STAT3 phosphorylation ($p<0.05$), showed an inverse modulation of circRNAs expression compared to IL-6 treatment.

Conclusions: This study reveals a T-LGLL-specific circRNA signature. neutropenic CD8+ *STAT3*-mutated patients showed a proper distinct circRNA profile, suggesting a role of these transcripts in disease severity and clinical manifestation. Experiments demonstrated that STAT3 activation drives circRNA dysregulation, offering new insights into T-LGLL pathogenesis. Future studies will explore circRNA function through siRNA silencing and RNA pull-down assays.