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blood advances

RPS15 mutations rewire RNA translation in chronic lymphocytic leukemia

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Key Points

- *RPS15* mutations rewire the translation program of primary CLL cells by reducing their translational efficiency.
- In the context of *RPS15* mutations, ribosomal proteins, regulatory elements, and immune signaling are regulated at the translational level.

Recent studies of chronic lymphocytic leukemia (CLL) have reported recurrent mutations in the *RPS15* gene, which encodes the ribosomal protein S15 (RPS15), a component of the 40S ribosomal subunit. Despite some evidence about the role of mutant RPS15 (mostly obtained from the analysis of cell lines), the precise impact of *RPS15* mutations on the translational program in primary CLL cells remains largely unexplored. Here, using RNA sequencing and ribosome profiling, a technique that involves measuring translational efficiency, we sought to obtain global insight into changes in translation induced by *RPS15* mutations in CLL cells. To this end, we evaluated primary CLL cells from patients with wild-type or mutant *RPS15* as well as MEC1 CLL cells transfected with mutant or wild-type *RPS15*. Our data indicate that *RPS15* mutations rewire the translation program of primary CLL cells by reducing their translational efficiency, an effect not seen in MEC1 cells. In detail, *RPS15* mutant primary CLL cells displayed altered translation efficiency of other ribosomal proteins and regulatory elements that affect key cell processes, such as the translational machinery and immune signaling, as well as genes known to be implicated in CLL, hence highlighting a relevant role for *RPS15* in the natural history of CLL.

Introduction

In chronic lymphocytic leukemia (CLL), we and others have reported recurrent mutations in the *RPS15* gene,[1-4] which encodes a component of the 40S ribosomal subunit.[1,2] *RPS15* mutations are infrequent (~4%) in untreated patients,[2,3] albeit enriched (~20%) within aggressive CLL, particularly in patients relapsing after treatment with fludarabine-cyclophosphamide-rituximab.[1] Interestingly, *RPS15* mutations were clonal before treatment and remained stable at relapse, pointing to an early event in CLL pathogenesis. In addition to the established role of ribosomal protein S15 (RPS15) in protein translation, evidence exists that RPS15 binds MDM2/MDMX, while its mutants reduce stabilization and increase p53 degradation, indicating a novel molecular mechanism involved in CLL pathobiology.[1]

A recent study reported that certain *RPS15* mutations affected both protein stability and cell proliferation rates in the fibroblast HEK293T and CLL MEC1 cell lines and that RPS15 variants induced proteome-wide changes.[5] Here, using ribosome sequencing (Ribo-seq), we investigated the possibility that defects in the synthesis or function of the ribosomes induced by *RPS15* mutations could also affect the pattern of translated messenger RNAs (mRNAs) in both primary CLL cells and CLL cell lines.

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RNA-seq and Ribo-seq data have been deposited to https://warehouse.inab.certh.gr/index.php/s/htsQNwllSop3vRN.

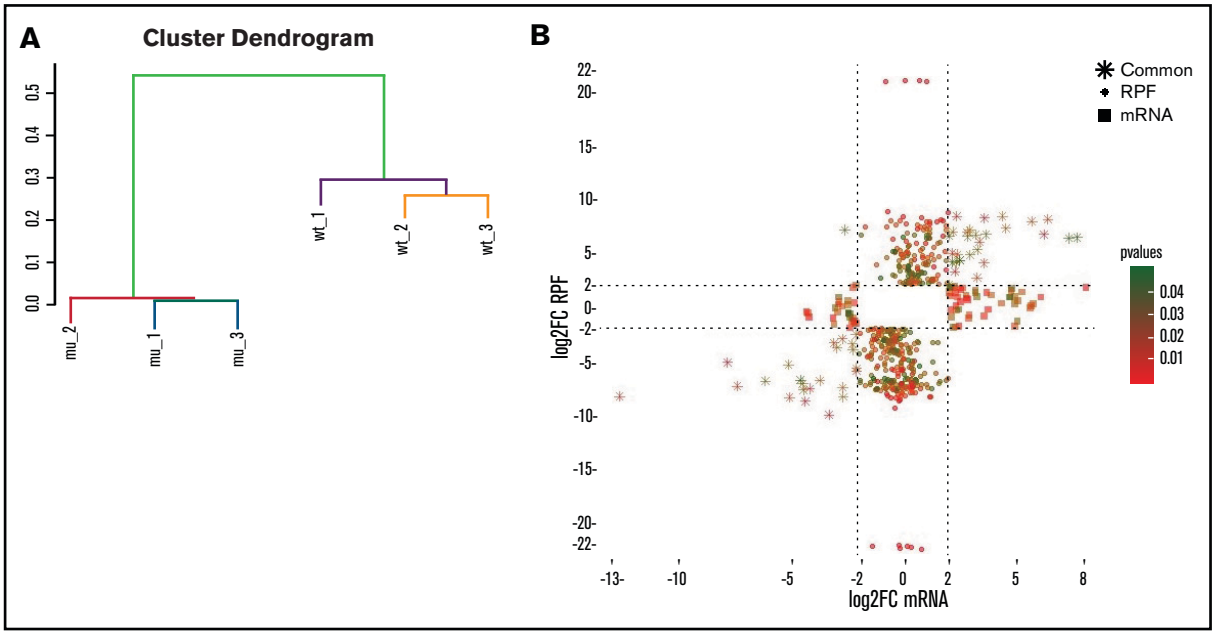

Figure 1. *RPS15* mutations mostly affect translation rather than transcription. (A) Cluster dendrogram based on unsupervised agglomerative hierarchical clustering analysis of the 4000 most variable genes (based on standard deviation). (B) Scatterplot indicating differentially expressed genes (log2FC≥|2|; $P < .05$) at mRNA and RPF level in *RPS15*^{mut} vs *RPS15*^{wt} CLL cases. The x-axis represents the log2FC of mRNAs, while the y-axis represents the log2FC of RPFs. The color code (green to red) indicates the statistical significance of the observed differences. Squares indicate genes that show differential expression at mRNA level only, circles indicate the genes that show differential expression at RPF level only, and stars indicate the genes that show differential expression at mRNA and RPF level.

Methods

Patient samples and generation of RPS15 mutant MEC1 cell lines

Blood samples were collected under informed consent from patients with CLL (supplemental Table 1). Additionally, transformed MEC1 CLL cells that overexpress mutant or wild-type RPS15 were generated as described in supplemental Methods.

The study was approved by the local ethics review committee of the participating institutions.

Ribosome profiling (Ribo-seq) and mRNA sequencing

Ribosome protected fragment (RPF) generation and library preparation for Ribo-seq and mRNA sequencing was performed using the TruSeq Ribo Profile (Mammalian) kit (Illumina) (supplemental methods). All samples were sequenced using Illumina's NextSeq-500 (with 75 nt read length). Each sequencing run resulted in 38 to 113 million raw reads/sample, of which 5 to 33 million and 13 to 79 million reads were used for subsequent analysis for RPFs and mRNAs, respectively (supplemental Table 2), following a bioinformatics pipeline described in supplemental Methods.

Flow cytometry

The protein expression of BCL2L11 and CXCR4 was assessed by flow cytometry as detailed in supplemental Methods.

Results and discussion

Ribo-seq involves measuring translational efficiency (TE), by comparing the levels of ribosome-associated mRNA footprints to the total mRNA for each gene.[6,7] Here, we applied Ribo-seq and RNA sequencing (RNA-seq) for studying primary CLL cells with wild-type *RPS15* (CLL_RPS15^{wt}) and mutant *RPS15* (CLL_RPS15^{mut}) (3 cases each), as well as mutant MEC1 (*RPS15*^{P131S} transfected MEC1_RPS15^{mut}) and wild-type MEC1 (*RPS15*^{wt} transfected MEC1_RPS15^{wt}) cells.

Ribo-seq revealed that (1) RPF libraries had the expected 28-nt size; (2) the reads from the RPF libraries were low at 3' untranslated regions, and a few RPFs mapped to annotated 5' untranslated regions, suggesting the presence of ribosomes at upstream open reading frames, while the majority mapped to coding regions; and (3) triplet periodicity was observed around the transcription start and stop sites. In contrast, the mRNA reads mapped uniformly across the length of the mRNA, as expected for randomly fragmented mRNA, with no triplet periodicity (supplemental Figure 1).

Unsupervised agglomerative hierarchical clustering analysis, based on the RPF normalized counts of the 4000 most variable genes, could distinguish *RPS15*^{mut} from *RPS15*^{wt} CLL cases (Figure 1A). In order to distinguish translational from transcriptional regulation of gene expression, we analyzed the Ribo-seq and mRNA-seq datasets separately and compared differences (log2 fold change [log2FC] $\geq |2|$, $P \leq .05$) in mRNA levels to variation in RPF abundance between CLL_RPS15^{wt} and CLL_RPS15^{mut}. We identified 3 groups of collectively 531 genes that represent different modes of regulation: (1) 404 genes with differences only at the translation (RPF) level, thus identified only by Ribo-seq analysis; (2) 74 genes with differences only at the transcription (mRNA) level, thus identified only by RNA-seq analysis; and (3) 53 genes with differences at both the transcription (mRNA) and translation (RPF) level, thus identified by both RNA-seq and Ribo-seq (Figure 1B). Altogether, these findings suggest

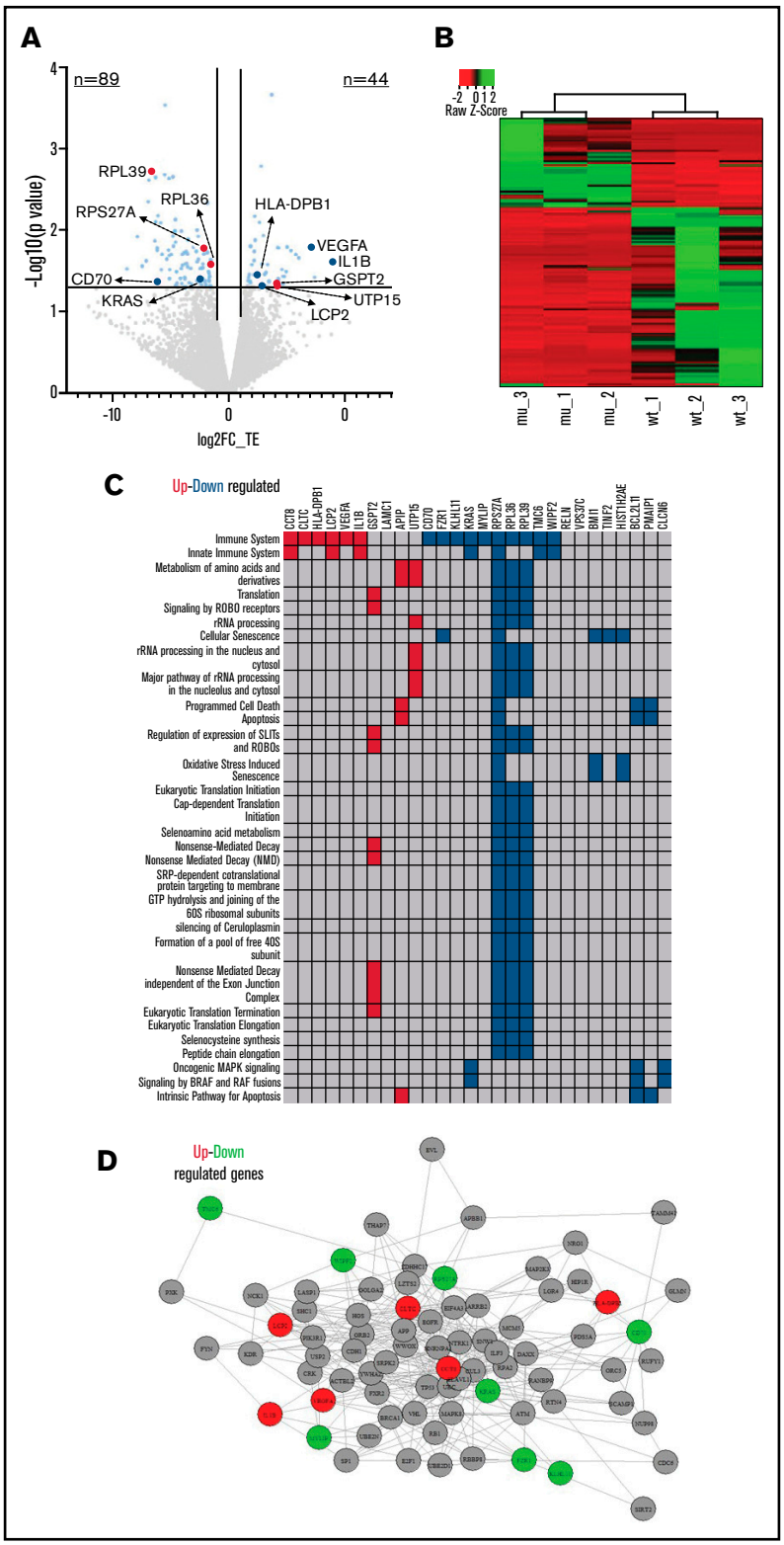

Figure 2. *RPS15* mutations rewire CLL translational program. (A) Volcano plot indicating the results from differential TE analysis in *RPS15*^{mut} vs *RPS15*^{wt} CLL cases. The x-axis represents the log2FC of TE, while the y-axis represents *P* values. Genes that show statistically significant differential TE (log2FC $\geq |1|$; $P < .05$) are highlighted in light blue. Genes highlighted in red are those implicated in the translation machinery, while genes in dark blue have already been implicated in CLL biology. (B) Supervised hierarchical clustering analysis based on the differentially translated genes between *RPS15*^{mut} and *RPS15*^{wt} CLL cases. (C) The figure shows enriched pathways from the Reactome database (false discovery rate < 0.05) and the differentially translated genes between *RPS15*^{mut} and *RPS15*^{wt} CLL cases. Upregulated genes in *RPS15*^{mut} compared with *RPS15*^{wt} cases are shown in red, while downregulated genes are shown in blue. (D) An example of the enriched immune signaling pathway. The interactions are confirmed by Biogrid. Upregulated genes in *RPS15*^{mut} compared with *RPS15*^{wt} cases are shown in red, while downregulated genes are shown in green.

that Ribo-seq can reveal additional information compared with mRNA-seq alone while also indicating that the predominant disruptive effect of *RPS15* mutations in CLL is exerted at the translation level.

In order to detect the major patterns of translational regulation, we calculated the differences in TE between *RPS15*^{mut} and *RPS15*^{wt} CLL cases. We found 133 genes with altered TE ($log2TE > |1|$, $P < .05$) of which 89 (66.9%) were downregulated in *RPS15*^{mut} CLL cases (Figure 2A; supplemental Table 3), implying that *RPS15* mutations mainly repress mRNA translation. Supervised hierarchical clustering analysis based on the differentially translated genes could discriminate the *RPS15*^{mut} from the *RPS15*^{wt} CLL cases (Figure 2B).

Functional enrichment analysis revealed that deregulated genes are implicated in processes relevant to translation such as translation initiation, elongation, and termination as well as ribosomal RNA processing, indicating that *RPS15* mutations affect key molecules of the translational machinery (Figure 2A). Moreover, immune processes were also affected; key deregulated molecules, already implicated in CLL biology, include CD70,[8] IL1B,[9] VEGFA,[10] KRAS,[11] HLA-DPB1[12] and LCP2[13] (Figure 2A,C-D; supplemental Table 4). Significant enrichment was found in processes related to protein binding, nucleic acid binding and catalytic activity on proteins (supplemental Table 5). Hence, many genes and processes associated with CLL and cancer at large are primarily translationally rather than transcriptionally deregulated in a setting of *RPS15* mutations.

We next performed similar analyses in MEC1 cells where more genes were found to show differences at the mRNA level only (n = 94), while fewer genes (n = 32) differed at the translation level. Relevant to mention, TE was induced in the majority of the genes (65.6%) in MEC1_RPS15^{mut} vs MEC1_RPS15^{wt} (supplemental Tables 6-7). The overlap between the differentially translated genes in primary CLL cells and the MEC1 cell line was minimal (0.7%) (supplemental Figure 2). The observed differences, may be attributed to the following reasons: (1) MEC1 cells are *TP53* aberrant; (2) MEC1_RPS15^{wt} cells overexpress the *RPS15* gene compared with primary CLL cells; and (3) MEC1 cells are highly proliferative, thus contrasting most circulating CLL cells and, moreover, are CD5 negative. Hence, results from CLL-derived cell lines, as those recently reported,[5] cannot be a priori considered as equivalent to those in primary CLL cells, since they appear dependent on the cell model used.

In order to validate Ribo-seq results, using flow cytometry, we assessed BCL2L11 protein expression in both *RPS15*^{mut} and *RPS15*^{wt} primary CLL cells (n = 13 and n = 8 cases, respectively), as it was found to exhibit remarkably reduced TE in the former. BCL2L11 was significantly downregulated in *RPS15*^{mut} samples (fold difference: 1.3; $P < .05$), in keeping with the reduced TE revealed by Ribo-seq in *RPS15*^{mut} vs *RPS15*^{wt} CLL cases (supplemental Figure 3A). We extended the validation experiments in *RPS15*^{mut} and *RPS15*^{wt} MEC1 cell lines, where we assessed the expression of CXCR4 by flow cytometry, found suppressed at the mRNA level in the former, and report concordant decreased CXCR4 protein expression in MEC1_*RPS15*^{mut} cells (fold difference: 1.95; $P = .1$) (supplemental Figure 3B).

In conclusion, our data indicate that *RPS15* mutations rewire the translation program of CLL cells by reducing their TE, likely due to altering the TE of other ribosomal proteins and regulatory elements, inducing substantial changes in several proteins implicated in CLL pathobiology. As the equation of transcript abundances to protein levels is still under consideration[14,15] we argue that the analysis of the translated genome in CLL will shed light into the genes and regulatory pathways implicated in disease phenotypes.

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Authorship

Contribution: S.N. designed the research, performed the study and wrote the manuscript; S.L., M.G., G.T. assisted in research; F.P., T.M., N.P., L.M. assisted in data analysis, and interpretation; A.A., N.S., S.P., K.P. provided patient samples; A.M. assisted in data interpretation and revised the manuscript; R.R. and K.S. designed and supervised the research and wrote the manuscript.

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