Differencial expression analysis of plasmatic long non-coding RNAs in patients with diffuse large B-cell lymphoma

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INTRODUCTION

DLBCL is the most common non-Hodgkin lymphoma in adults. Long non-coding RNAs (lncRNAs) represent a heterogeneous group of protein-non-coding RNAs with a length of >200 nucleotides, whose function affects a number of physiological and pathological processes. Due to the short life of RNA in the blood, their representation reflects the patient's current condition, and thanks to the high tissue specificity, lncRNAs from liquid biopsy can be used as biomarkers for monitoring the treatment effect.

METHODS

Patients' plasma is obtained by centrifugation of whole, (K_3EDTA) non-clotting blood within 1 hour after collection. cfRNA (extracellular RNA = cell-free RNA) is subsequently isolated and quantified. After that, 3' polyadenylated sequencing libraries are prepared, which are then analyzed on a new generation sequencer (see Figure 1). Sequencing data is analysed by bioinformatic pipeline (see Figure 2).

WORKFLOW

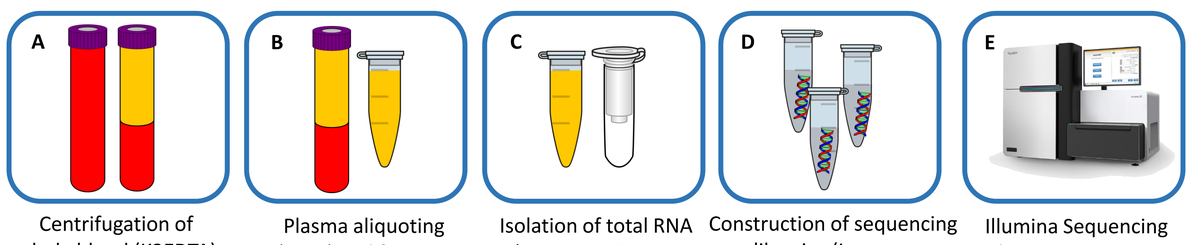

Fig. 1: Diagram of plasma sample collection (A), biobanking (B), RNA isolation (C), library preparation (D) and sequencing (E).

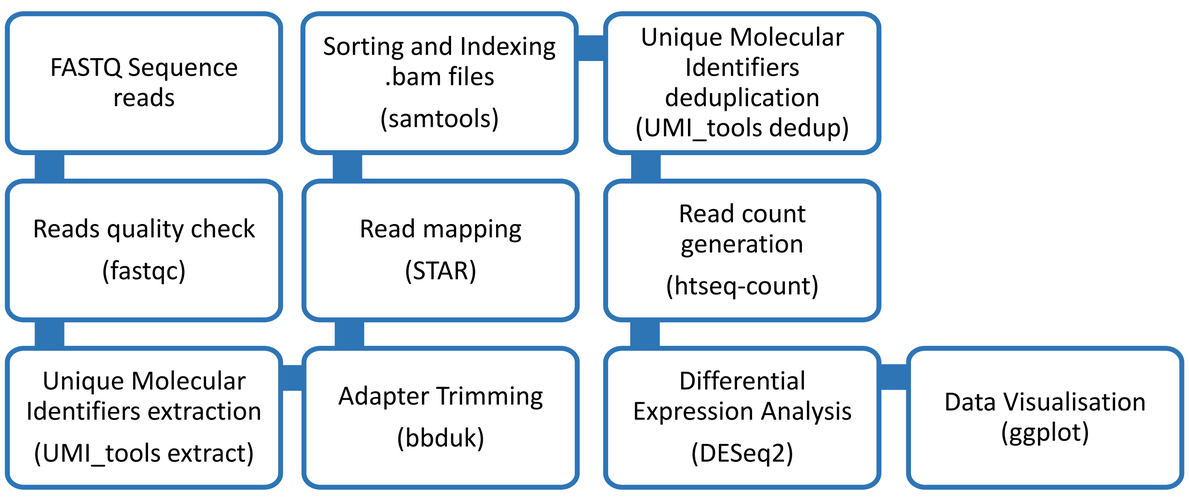

Fig. 2: Structure of the bioinformatic pipeline

RESULTS

Three independent cfRNA sequencing experiments were performered from a total of 30 DLBCL patients before and after treatment. Analysis across all samples revealed 8425 genes with CPM (counts per milion) > 1000, of which 2319 were identified as lncRNA. Regardless of gender or DLBCL cell-type (GCB, ABC) the analysis identified 15 differentially expressed lncRNA. Data visualisation shows two overexpressed genes, lnc-CERKL-2 and CCDC26, which was previously described as a predictive biomarker for acute myeloid leukemia[1]. In only three patients this gene was not overexpressed before treatment. Down-expressed genes are, for example, lncRNA COMETT, which induces MAPK and PI3H/AKT signaling pathways and anti-sense lncRNA to SMAD3 gene, regulating TGF-ß mediated transcription and intracellular signaling.

Gene stable ID	Gene name	Gene type	Gene description
ENSG00000226856	THORLNC	lncRNA	testis associated oncogenic lncRNA [Source:HGNC Symbol;Acc:HGNC:53788]
ENSG00000259347	Lnc-AAGAB-1	lncRNA	novel transcript, antisense to SMAD3
ENSG00000230876	LINC00486	lncRNA	long intergenic non-protein coding RNA 486 [Source:HGNC Symbol;Acc:HGNC:42946]
ENSG00000214870	LINC02981	lncRNA	long intergenic non-protein coding RNA 2981 [Source:HGNC Symbol;Acc:HGNC:56055]
ENSG00000250723	Lnc-DTHD1-5	lncRNA	novel transcript
ENSG00000254689	LINC02235	lncRNA	long intergenic non-protein coding RNA 2235 [Source:HGNC Symbol;Acc:HGNC:53106]
ENSG00000224970	HSALNG0061925	lncRNA	novel transcript, antisense to TRPV6
ENSG00000247134	Lnc-NRG1-2	lncRNA	novel transcript
ENSG00000227028	SLC8A1-AS1	lncRNA	SLC8A1 antisense RNA 1 [Source:HGNC Symbol;Acc:HGNC:44102]
ENSG00000238121	LINC00426	lncRNA	long intergenic non-protein coding RNA 426 [Source:HGNC Symbol;Acc:HGNC:42761]

Fig. 3: List with the ten most expressed lncRNA genes in pre-treatment plasma samples.

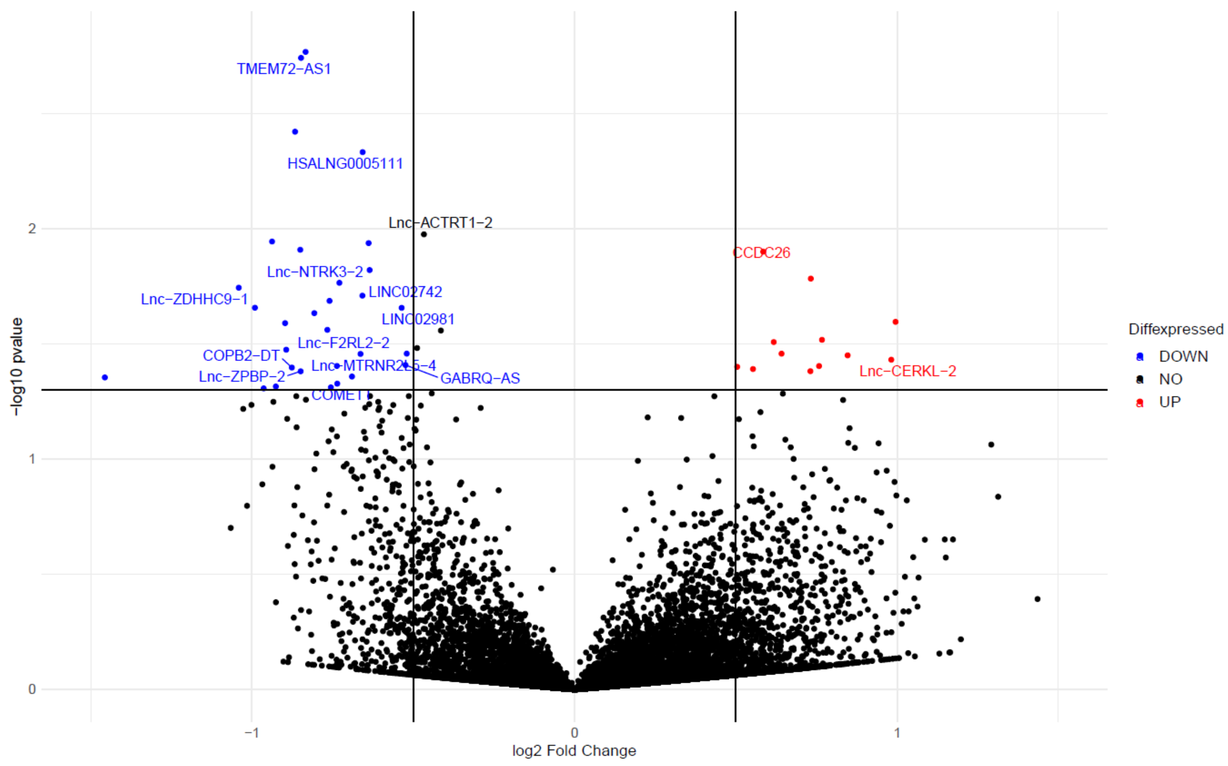

Fig. 4: Volcano plot representing differentially expressed genes in pre-treatment samples with a p-value < 0.05 and log2 fold change > 0.5. Overexpressed genes are shown in red on the right and downexpressed gene in blue on the left. Only lncRNAs using Biomart are annotated.

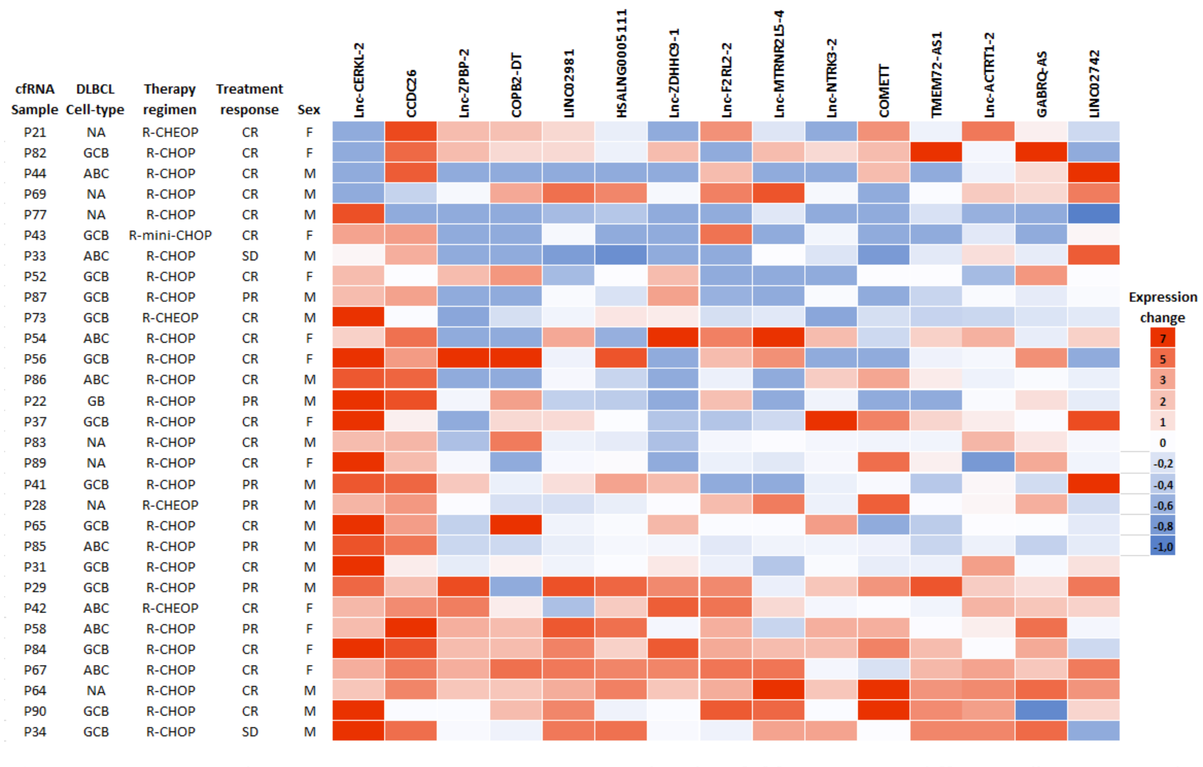

Fig. 5: Heat map, showing pre-treatment expression levels of fifteen most differentially expressed lncRNA genes across all thirty samples.

CONCLUSION

lncRNAs can be used to monitor cancer or as a biomarkers for an early detection of various malignancies. In general, lncRNAs obtained from liquid biopsies represent potential for tumor detection, monitoring and diagnosis. Our research will now focus on further data analysis, finding RNA editing sites, mutations and our data will be validated on measured dataset using RT-qPCR.

FUNDING

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[1] Chen C, Wang P, Mo W, et al. lncRNA-CCDC26, as a novel biomarker, predicts prognosis in acute myeloid leukemia. *Oncol Lett.* 2019;18(3):2203-2211. doi:10.3892/ol.2019.10591