RNA-seq Insights into Major Regulatory Networks in Pediatric Leukemia

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Abstract

Pediatric leukemia is a leading cause of cancer-related mortality in children, necessitating a deeper understanding of its molecular mechanisms. This study identifies key regulatory networks driving pediatric leukemia using RNA sequencing (RNA-seq) and data from the European Nucleotide Archive (ENA). Peripheral blood samples from pediatric leukemia patients and healthy controls were analyzed. The computational pipeline included quality control via FastQC, read alignment to the reference genome using STAR and differential gene expression analysis with DESeq2. Total 250 differentially expressed genes (DEGs) were identified between leukemia and control samples. Key pathways associated with leukemogenesis including cell cycle regulation, apoptosis and immune response, were significantly enriched. Transcription factors such as MYC and NF-κB were highlighted as central regulators of these networks. Gene ontology (GO) and pathway enrichment analysis were performed using DAVID and KEGG databases, revealing dysregulated immune signaling as a prominent feature of pediatric leukemia. Weighted gene co-expression network analysis (WGCNA) was employed to identify gene modules strongly correlated with leukemic phenotypes.

This study provides a comprehensive overview of dysregulated gene networks in pediatric leukemia, leveraging publicly available ENA data and advanced computational techniques. The results offer potential biomarkers for early diagnosis and new therapeutic targets in pediatric leukemia, contributing to a better understanding of its molecular landscape.

Keywords: RNAseq, pediatric leukemia, computational analysis, DEG, Cancer.

Introduction

Pediatric leukemia is the most common form of childhood cancer, accounting for approximately one-third of all cancer diagnoses in children[3]. Among the subtypes, acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) are the most prevalent[21,22]. Despite advancements in treatment such as chemotherapy and stem cell transplantation, relapse remains a significant challenge, particularly in high-risk patients[5]. Relapsed pediatric leukemia often exhibits aggressive behaviour, drug resistance and poor prognosis, which necessitate a deeper understanding of its molecular underpinnings[10].

Pediatric leukemia is the most common childhood cancer, accounting for approximately 30% of all pediatric cancer diagnoses[2]. There are two primary types: acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML)[9,11]. Despite significant advances in treatment, leukemia remains a leading cause of cancer-related deaths in children, especially when relapse occurs[4]. Over the years, research has shifted towards uncovering the molecular and genetic factors that contribute to leukemia onset, progression and relapse, with the aim of developing more targeted and effective therapies[12].

Current research in pediatric leukemia is largely focused on identifying genetic mutations and dysregulated pathways that can be exploited for treatment[17]. Studies have identified mutations in genes like *UBTF* in AML which affect the transcription process, leading to aggressive forms of leukemia. Similarly, in pediatric ALL, rearrangements in genes like *LMO2* and *STAG2* have been found to predict extremely high-risk subtypes, particularly in T-cell ALL[23]. The symptoms of pediatric leukemia are often nonspecific, making early diagnosis challenging. Common symptoms include persistent fatigue, frequent infections, unexplained bruising, bone pain and anaemia. Many children also have swollen lymph nodes, hepatosplenomegaly (enlarged liver and spleen) and bleeding tendencies due to low platelet counts.

The diagnosis of pediatric leukemia involves a combination of blood tests, bone marrow biopsies and genetic profiling. Blood tests typically reveal abnormalities in white blood cell counts, anaemia and thrombocytopenia[24]. Bone marrow biopsies are critical for confirming the presence of leukemic blasts. Cytogenetic and molecular analyses are increasingly used to identify specific genetic mutations and chromosomal abnormalities, providing vital information for prognosis and treatment decisions[25]. The identification of molecular markers through diagnostic tests like next-generation sequencing allows for better risk stratification and the development of personalized treatment plans including targeted therapies and immunotherapies. These advances are crucial in improving outcomes, particularly for high-risk and relapsed pediatric leukemia cases[26].

Recent advancements in high-throughput sequencing technologies such as RNA sequencing (RNA-seq) have revolutionized our ability to study cancer at a transcriptomic level. RNA-Seq provides a powerful platform to analyze the

gene expression profiles of both normal and cancerous cells, enabling the identification of differentially expressed genes (DEGs) that may play a critical role in disease progression, relapse and therapeutic resistance[13].

This study aims to employ RNA-Seq to identify key genes and pathways involved in relapsed pediatric leukemia[14]. By comparing the transcriptomic profiles of relapsed and non-relapsed leukemia samples, we seek to uncover potential biomarkers for early relapse detection and identify novel therapeutic targets that could improve treatment outcomes for pediatric leukemia patients[27].

Material and Methods

The dataset utilized in this study was sourced from the Gene Expression Omnibus (GEO) under accession number GSE266550 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE266550. The dataset utilized in this study comprises of RNA sequencing (RNA-Seq) data aimed at identifying cryptic gene fusions among specific subcohorts of patients diagnosed with acute lymphoblastic leukemia. This analysis focuses on both relapsed and non-relapsed patients including samples from three patients who experienced relapse and eight patients who remained non-relapsed.

Comparative analysis between relapsed and non-relapsed allows for the identification of Differentially expressed gene (DEG)) and molecular differences underlying treatment outcomes in these patient groups. The insights gained from this dataset contribute to a better understanding of the genetic mechanisms associated with acute lymphoblastic leukemia. Differentially expressed gene (DEG) analysis is a vital approach in genomics that compares gene expression levels between different conditions, such as diseased versus healthy samples. Steps for DEG analysis

1. **Data Acquisition:** Gene Expression Omnibus (GEO) under accession number GSE266550 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE266550.
2. **Preprocessing:** Quality control, normalization and transformation to ensure accuracy and comparability.
3. **Statistical Analysis:** Statistical tests DESeq2 was applied to identify genes with significant expression changes across conditions. DEGs are filtered based on fold change and p-values to determine biological relevance.
4. **Functional Annotation:** Identified DEGs were analyzed for their biological functions and pathways using databases such as Gene Ontology (GO) or KEGG, revealing insights into the underlying biological processes.
5. **Interpretation and Validation:** The results are interpreted in the context of the biological question and validation may be performed through techniques like qPCR or Western blotting to confirm findings.

Results and Discussion

Differentially Expressed Genes analysis: The boxplot illustrated in the figure 1 displays the normalized RNA-seq counts (log10 scale) for samples from the dataset GSE266550, comparing relapsed and non-relapsed patients. The green boxes represent the relapsed patient cohort while the purple boxes depict the non-relapsed cohort. Each box encompasses the interquartile range (IQR), with the line inside each box indicating the median value. The whiskers extend to the minimum and maximum values, reflecting the overall distribution of normalized counts for each sample. Boxplot shows the differences in gene expression profiles between relapsed and non-relapsed patients which show the molecular variations associated with treatment outcomes.

Table 1 presents the results of a differential gene expression analysis comparing relapsed and non-relapsed samples. It includes key metrics such as the geneID, gene symbol, a brief description of each gene, the logarithm (base 10) of the mean expression levels, the logarithm (base 2) of the fold change in expression between the two groups and the negative logarithm (base 10) of the P-value, indicating the statistical significance of the findings.

Log10 base mean represents the mean expression level of the gene, expressed on a logarithmic scale (base 10), which helps in comparing the expression levels across genes.

Table 1
Differential gene expression analysis comparing relapsed and non-relapsed samples

GeneID	Symbol	Description	log10 base Mean	log2 (fold change) (Relapsed vs Non Relapsed)	-log10(Pvalue) (Relapsed vs Non Relapsed)
911	CD1C	CD1c molecule	2.232	4.919	2.576
166348	KBTBD12	kelch repeat and BTB domain containing 12	1.512	4.124	1.309
221711	SYCP2L	synaptonemal complex protein 2 like	2.185	4.527	1.309
8972	MGAM	maltase-glucoamylase	1.731	2.517	2.576
5657	PRTN3	proteinase 3	2.215	4.539	1.309
4744	NEFH	neurofilament heavy chain	1.429	3.053	1.309

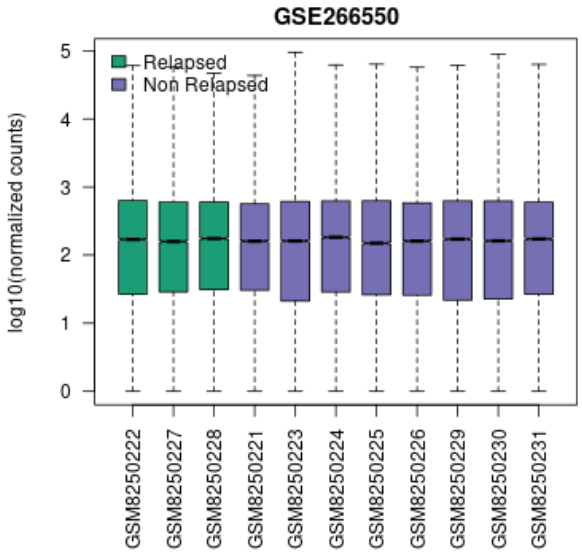

Figure 1: Normalized RNA-seq counts (log10 scale) for samples from the dataset GSE266550, comparing relapsed and non-relapsed patients.

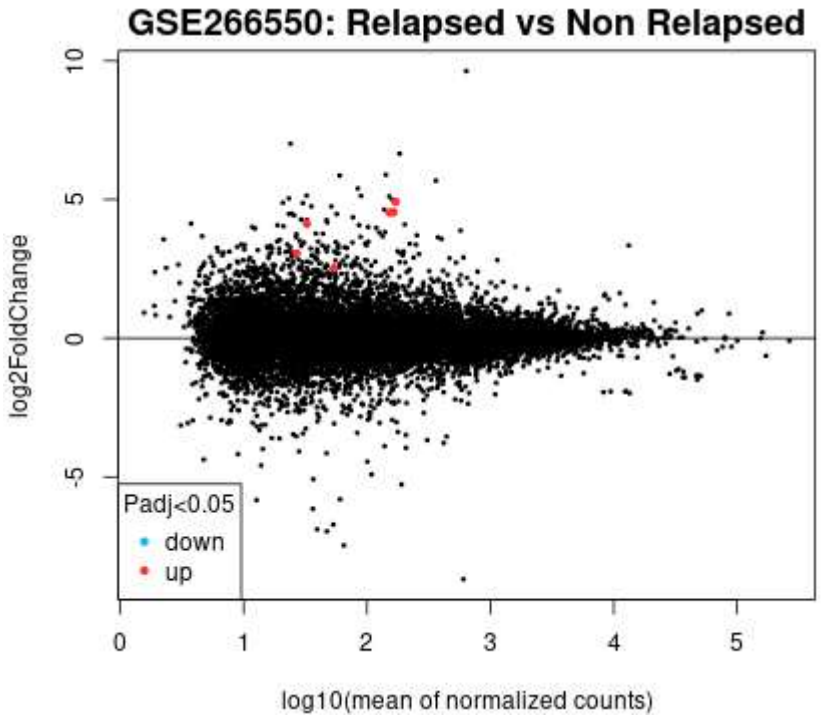

Figure 2: Differential Expression Analysis of GSE266550 (Relapsed vs Non-Relapsed Samples).

log2(fold change) (Relapsed vs Non Relapsed) gives the fold change in expression between relapsed and non-relapsed samples, calculated on a logarithmic scale (base 2). A positive value indicates higher expression in relapsed samples, while a negative value suggests higher expression in non-relapsed samples. -log10(P-value) (Relapsed vs Non Relapsed) shows the statistical significance of the observed differences in expression, with higher values indicating greater significance.

The genes listed in table 1 show varying degrees of differential expression with notable candidates such as CD1C[18] and MGAM[20], which may play significant roles in the pathology of relapse in the studied condition. These expression changes can provide insights into the molecular mechanisms underlying cancer relapse and may inform future therapeutic strategies. MA plot as shown in figure 2 visualizes the log2 fold changes (y-axis) versus the mean of normalized counts (x-axis) for gene expression in relapsed versus non-relapsed conditions. Each point represents a gene with significantly differentially expressed genes (adjusted p-value < 0.05) highlighted in red (upregulated) and blue (downregulated). Most genes cluster around a log2 fold change of zero, indicating no significant difference, while outliers represent genes with notable up- or down-regulation in relapsed samples.

Volcano plot as shown in figure 3 represents the relationship between statistical significance (y-axis, -log10 of adjusted p-values) and fold change (x-axis, log2FC) for gene expression in relapsed versus non-relapsed samples. Red dots indicate significantly upregulated genes (adjusted p-value < 0.05), while blue dots would signify downregulated genes (none are visible here). The majority of points are clustered near log2FC = 0, indicating little to no change in expression, with a few genes showing substantial upregulation in relapsed samples.

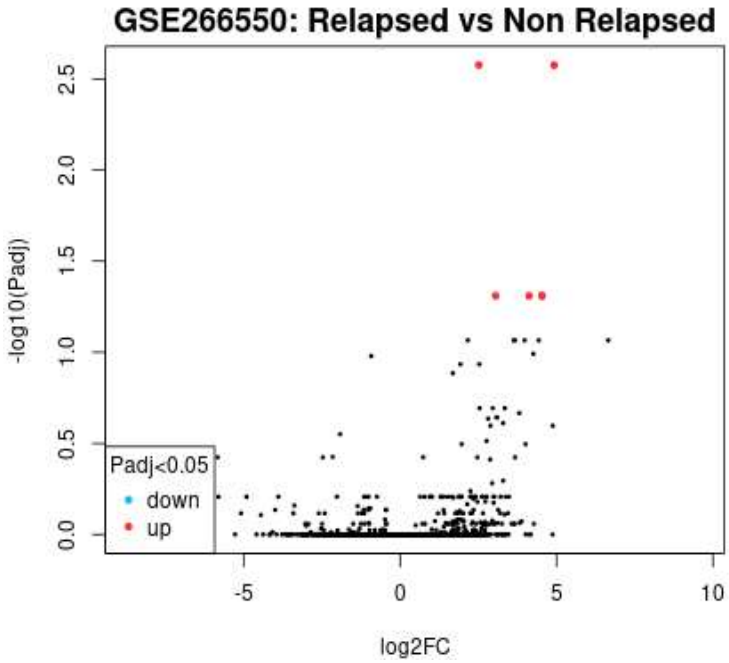

Figure 3: Volcano Plot of GSE266550 (Relapsed vs Non-Relapsed Samples).

Table 2
Functional annotation of DEGs along with their key pathways, functions and associations with various diseases.

S.N.	Gene	Key Pathways	KEGG Pathway ID	Function	Disease Association
1	CD1C (CD1c molecule)	Antigen presentation pathway and Immune response via lipid and glycolipid presentation to T cells	hsa04660 (T cell receptor signaling)	Presents lipid and glycolipid antigens to T cells, activating immune responses	Autoimmune diseases (e.g., Type 1 diabetes, sarcoidosis)
2	SYCP2L (Synaptonemal complex protein 2 like)	Meiotic recombination	hsa04114 (Oocyte meiosis)	Involved in chromosome synapsis and meiotic recombination during spermatogenesis and oogenesis	Variants potentially linked to infertility and meiosis-related syndromes
3	MGAM (Maltase-glucoamylase)	Carbohydrate digestion and absorption	hsa04973 (Carbohydrate digestion and absorption)	Catalyses the final steps of starch digestion by breaking down maltose and glucose polymers	Deficiencies associated with congenital sucrase-isomaltase deficiency (CSID)
4	PRTN3 (Proteinase 3)	Neutrophil degranulation	hsa04657 (Neutrophil extracellular trap formation)	Serine protease involved in the breakdown of proteins during immune and inflammatory responses	Strongly associated with granulomatosis with polyangiitis (Wegener's granulomatosis), vasculitis
5	NEFH (Neurofilament heavy chain)	Neuronal cytoskeleton organization	hsa05020 (Prion diseases)	Major structural component of the neuronal cytoskeleton, stabilizes axons	Mutations linked to amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth disease

Gene Function and Pathway analysis: Gene function and pathway analysis of differentially expressed genes (DEGs) are essential for understanding the underlying biological mechanisms driving specific phenotypes or diseases. By identifying DEGs through techniques like RNA sequencing or microarrays, researchers can assess their roles in critical cellular processes, such as signal transduction, metabolic regulation and immune responses. Utilizing bioinformatics tools, pathways can be mapped using databases like KEGG or Reactome, enabling the elucidation of interaction networks among genes. This analysis not only reveals potential biomarkers for disease but also identifies therapeutic targets, paving the way for innovative treatment strategies and personalized medicine approaches in clinical settings.

Table 2 outlines several important genes along with their key pathways, functions and associations with various diseases. Each gene plays a crucial role in biological processes and can be linked to specific health conditions. Key pathways indicates the primary biological pathways in which the gene is involved, highlighting its role in cellular processes. Function describes the specific biological role of the gene product such as enzyme activity or structural functions. Disease association lists diseases or conditions linked to the gene, either through mutations or expression changes, indicating its clinical relevance.

CD1C (CD1c molecule)[1] gene is crucial for the antigen presentation pathway, particularly in presenting lipid and glycolipid antigens to T cells, thereby activating immune responses. It is associated with autoimmune diseases such as type 1 diabetes and sarcoidosis[8]. SYCP2L (Synaptonemal complex protein 2 like) is involved in meiotic recombination. This gene plays a significant role in chromosome pairing during meiosis. Variants of this gene may be linked to infertility and other meiosis-related syndromes. MGAM (Maltase-glucoamylase) enzyme is a key in carbohydrate digestion, catalysing the breakdown of starch[15]. Deficiencies in this enzyme can lead to congenital sucrase-isomaltose deficiency (CSID). PRTN3 (Proteinase 3) is a serine protease involved in neutrophil degranulation. This gene has strong associations with granulomatosis with polyangiitis, a form of vasculitis, highlighting its role in inflammatory responses[16].

NEFH (Neurofilament heavy chain)[6] is critical for maintaining neuronal structure. Mutations in this gene are linked to neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth disease, indicating its importance in neuronal health[7,19].

Conclusion

In conclusion, this study provides valuable insights into the molecular mechanisms underpinning pediatric leukemia, particularly focusing on differentially expressed genes (DEGs) between relapsed and non-relapsed patients. Through RNA sequencing and comprehensive bioinformatics analysis, several key genes such as CD1C, SYCP2L, MGAM, PRTN3 and NEFH were identified as significantly differentially expressed. These genes are involved in critical biological pathways such as immune response, carbohydrate digestion, neutrophil degranulation and neuronal cytoskeleton organization.

The results demonstrate that dysregulated immune signaling and the involvement of specific genes, particularly in the antigen presentation and meiotic recombination pathways, may play a crucial role in leukemia relapse. This study also highlights potential biomarkers like CD1C for early diagnosis and therapeutic targeting, offering a path for personalized medicine approaches in pediatric leukemia treatment. By leveraging publicly available RNA-seq data and advanced computational tools, this research provides a comprehensive view of gene networks associated with leukemia relapse. These findings contribute significantly to our understanding of leukemia's molecular landscape and could pave the way for future research aimed at improving clinical outcomes.

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