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Proceeding Paper

RUNX1-Regulated Pathways and Biomarkers in Acute Myeloid Leukaemia †

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Abstract: Runt-related transcription factor 1 gene (*RUNX1*), also known as acute myeloid leukaemia 1 protein (AML1), plays a crucial role in the pathogenesis of AML. RUNX1/AML1 is one of the most frequently mutated leukaemias associated with a poor prognosis in AML. Researchers and clinicians can develop personalized medicines and improve diagnosis by identifying the biomarkers associated with mutations. In the current study, we used the genome and transcriptome data from The Cancer Genome Atlas-Acute Myeloid Leukemia (TCGA-AML) cohort. We analysed *RUNX1* mutant AML patients compared to non-mutant patients using an integrated multi-omics, multi-database analysis of exome, and transcriptomics data. Finally, we identified the gene signature associated with *RUNX1* mutations, including prognostic genes that significantly influenced the overexpression of *RUNX1* mutation-associated genes in AML patients. Our results can help to diagnose AML patients with *RUNX1* mutations at an early stage.

Keywords: RUNX1; acute myeloid leukaemia; biomarkers; multi-omics

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1. Introduction

Acute myeloid leukaemia (AML) is a malignant hematological disease affecting the blood and bone marrow. RUNX1 (also known as AML1) is a transcription factor that plays an important role in blood cell development and function [1]. Mutations in the *RUNX1* gene have been linked to several blood disorders, including AML, and are associated with a poor prognosis. *RUNX1* mutations can lead to the cause of familial platelet disorder (FPD). Researchers are actively studying RUNX1 and its role in blood disorders with the aim of developing more effective treatments [2]. This includes the development of targeted therapies that specifically target abnormal blood cells produced by *RUNX1* mutations, as well as the development of new strategies to restore the *RUNX1* gene to normal function. In this study, we used TCGA-AML [3] data in which *RUNX1* is mutated in 9% of patients and identified the prognostic biomarkers specific to the *RUNX1* mutation.

2. Methods

2.1. Identification of Mutational Landscape of RUNX1 in TCGA-AML

The cBioPortal for Cancer Genomics website was used to identify the *RUNX1* mutational landscape in AML patients from the TCGA study (n = 200) [3–5].

2.2. Analysis of Differentially Expressed Genes (DEGs) in RUNX1-Mutated TCGA-AML

Out of 200 TCGA-AML patients, only 173 patients' RNA-Seq data were available. Based on the *RUNX1* mutations of TCGA-AML, we stratified the total number of patients

into two groups and designated them as *RUNX1*-mutated (n = 17) (Table S1) and wild-type (n = 156) (without *RUNX1* mutations), respectively. The mRNA expression profiles (RNA Seq V2 RSEM) were checked to identify the DEGs in these two groups. From the list of DEGs, we can conclude that they are the driving genes behind tumorigenesis and cancer progression.

2.3. Functional Annotation and Survival Analysis

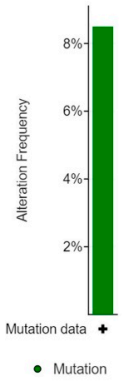
The functional annotation of the DEGs from *RUNX1*-mutated patients was performed by a web tool named DAVID [6]. This analysis provides the Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway information for genes.

The GEPIA2 tool [7] was used to evaluate the prognostic value of the DEGs identified in the patients with the *RUNX1* mutation in the TCGA-AML cohort. Briefly, for the TCGA-AML cohort, the patient samples are divided into two risk groups such as low-risk and high-risk groups, and the log-rank test, also known as the Mantel–Cox test, was performed to construct the overall survival plots. A p-value cut-off of <0.05 was used as the significance threshold in the search for prognostic biomarkers.

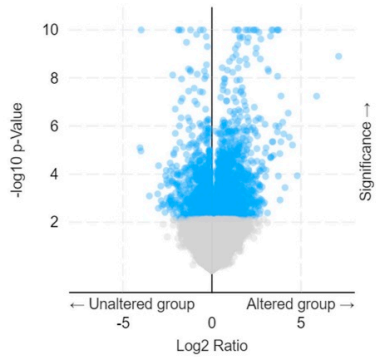
3. Results and Discussions

In TCGA-AML, *RUNX1* mutations occurred in 9% of patients (n = 17) out of a total of 200 patients (Figure 1A and Table S1). We then performed the DEGs analysis between the *RUNX1*-mutated vs. wild-type patients using cBioPortal. As a result, we obtained a total of 210 DEGs containing 155 upregulated and 55 downregulated genes in *RUNX1*-mutated patients with a fold change (FC) threshold of >2 and a p-value and q-value of <0.05 (Figure 1B and Table S2).

A



B



C

KEGG Pathways		
Term	*Count*	*p value*
hsa05200:Pathways in cancer	14	0.000157501
hsa04510:Focal adhesion	8	0.000825502
hsa04371:Apelin signaling pathway	6	0.004151938
hsa02010:ABC transporters	4	0.004866217
hsa05222:Small cell lung cancer	5	0.005325147
hsa04630:JAK-STAT signaling pathway	6	0.008718965
hsa05145:Toxoplasmosis	5	0.01056525
hsa05205:Proteoglycans in cancer	6	0.020196821
hsa01522:Endocrine resistance	4	0.039193786
hsa04640:Hematopoietic cell lineage	4	0.04020315
hsa05146:Amoebiasis	4	0.043310188

Figure 1. (**A**) Percentage of *RUNX1* mutations in TCGA-AML patients. (**B**) Volcano plot showing the DEGs between *RUNX1*-mutated vs. wild-type patients. Blue dots are significant differentially expressed genes whereas grey dots are not significant. (**C**) KEGG pathway analysis of upregulated genes in *RUNX1*-mutated patients.

Next, we selected DEGs, and then performed functional annotation analysis with DAVID separately for up- and downregulated genes. Interestingly, the KEGG analysis of upregulated genes obtained 10 pathways with a stringent p-value cut off of <0.05, in which the majority of genes are involved in pathways in cancer, focal adhesion, apelin signalling pathway, ABC transporters, small cell lung cancer, JAK-STAT signalling pathway, toxoplasmosis, proteoglycans in cancer, endocrine resistance, hematopoietic cell lineage and amoebiasis (Figure 1C and Table S3).

Next, we focused on the KEGG pathway analysis (p-value < 0.05) of downregulated genes, from which we obtained only one pathway identifying the genes involved in Staphylococcus aureus infection (Table S3). Overall, the functional annotation analysis

showed that the genes that are upregulated in the *RUNX1*-mutated patients are involved in a variety of signalling pathways that drive AML.

Our next goal was to examine whether these upregulated genes in *RUNX1*-mutated patients play a role in the prognosis of AML patients. Using the GEPIA2 web tool, we identified seven poor prognostic biomarkers whose significantly higher expression (p-value < 0.05) results in poor overall survival in TCGA-AML patients (Figure 2A). The genes with poor prognosis identified in our study are EGFEM1P, DOCK1, HTR1F, CALCRL, HOPX, TRIM9 and MYLK. These results clearly indicate that the increased expression of genes associated with *RUNX1* mutations acts as a biomarker in AML patients. We considered these seven genes to be *RUNX1* mutation-associated gene signatures (RMAGS) in AML. Notably, the higher expression of two downregulated genes such as KCNE5 and ROPN1L showed a good prognosis in AML patients (Figure 2B,C).

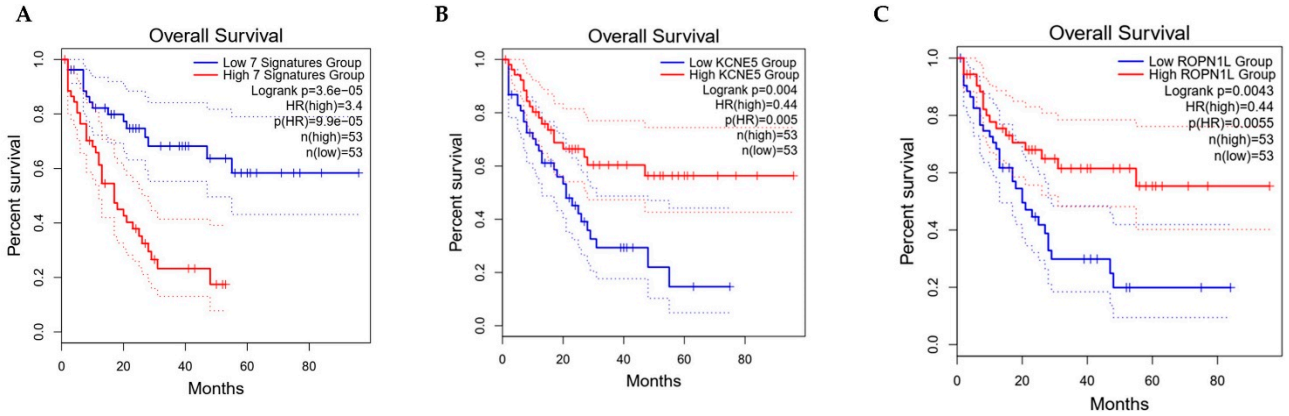


Figure 2. The survival plots showing the overall survival analysis of the seven gene signatures (RMAGS) along with the two downregulated genes (KCNE5 and ROPN1L) of *RUNX1* mutated TCGA-AML patients; (**A**) high expression of RMAGS indicates poor survival; (**B**,**C**) higher expression of KCNE5 and ROPN1L indicates good prognosis.

4. Conclusions

Taken together, our results identified a list of genes that are associated with the *RUNX1* alterations in AML patients, and we named them RMAGS. The increased expression of RMAGS predicts poor survival in AML patients. These seven genes may act as prognostic biomarkers individually and combinedly and can be used to identify the *RUNX1* mutation status in AML patients. In summary, our identified RMAGS could be possible targets in the treatment of AML, in that the development of combined inhibitors for this gene signature, along with RUNX1, could pave the way for the development of personalized/precision medicine to suppress RUNX1-mediated tumour growth and drug resistance.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/IECC2023-14279/s1; Presentation: RUNX1-regulated pathways and biomarkers in Acute Myeloid Leukaemia; Table S1: Summary table showing the detailed mutation information of *RUNX1* and its mRNA expression in TCGA-AML patients; Table S2: List of genes up and down regulated in *RUNX1* mutated AML patients; Table S3: Functional annotation analysis of up and down regulated genes associated with *RUNX1* mutations in AML.

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