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RUNX1-regulated pathways and biomarkers in Acute Myeloid Leukemia

Deepesh Kumar Verma[1], Akhileshwar Namani[2]

1. **Department of Biotechnology, GITAM School of Sciences, GITAM (Deemed to be University), Visakhapatnam, 530045, India**

2. **Sri Shankara Cancer Hospital and Research Centre, Bangalore, India**

- # Abstract
- Runt-related transcription factor 1 gene (RUNX1), also known as acute myeloid leukemia 1 protein (AML1), plays a critical role in the pathogenesis of AML. One of leukemia's most frequently mutated genes is RUNX1/AML1, which is related to a poor prognosis in AML. Researchers and clinicians can design personalized medicines and enhance diagnosis by identifying biomarkers linked to genetic mutations. In the current study, we used TCGA-Acute Myeloid Leukemia (AML) cohort's genomic and transcriptome data. We analyzed RUNX1 mutated AML patients to non-mutated patients using an integrated, multi-omics, multi-database analysis of exome and transcriptomics data. The mutation landscape of several genes, including RUNX1 mutations, was revealed by TCGA-AML data from multi-center high-throughput exome sequencing. Finally, we identified the gene signature associated with RUNX1 mutations, including prognostic genes that significantly impacted the overexpression of the RUNX1 pathway in RUNX1 mutated AML patients. Our findings may help diagnose AML patients with RUNX1 mutations early.

- **Keywords:** RUNX1, AML, acute myeloid leukemia, biomarkers, multi-omics, blood cancer.

1. Introduction

- Acute myeloid leukemia (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 prog- nosis. 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 [4] databases for cancer genomics website was used to identify the *RUNX1* mutational landscape in AML patients from the TCGA study (n =200) [3].

2.2 Analysis of differentially expressed genes (DEG's) in RUNX1 mutated TCGA-AML

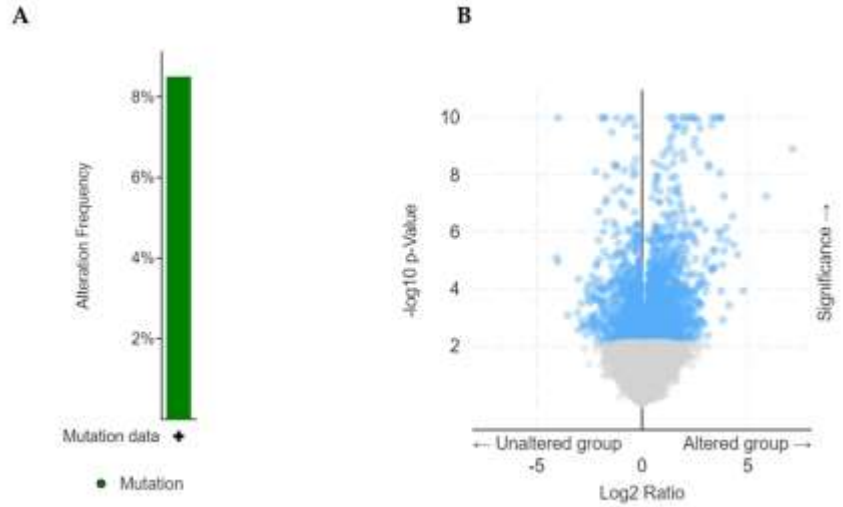
- Based on the *RUNX1* mutations of TCGA-AML, we stratified the total number of patients into two groups and designated them as *RUNX1*-mutated (n=17) and wild-type (n=183)(without *RUNX1* mutations), respectively. The mRNA expression profiles (RNA Seq V2RSEM) were checked to identify the DEG's in these two groups. From the list of DEG's, 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 DEG's from *RUNX1* mutated patients was performed by a web tool named DAVID [5]. This analysis provides the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway information for genes. The GEPIA2 tool [6] was used to evaluate the prognostic value of the DEG's identified in the patients with *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. (p-value cutoff <0.05)

3. Results

- In TCGA-AML, RUNX1 mutations occurred in 9% of patients (n=17) out of 200 patients (Figure 1 A). We then performed the DEG's analysis between RUNX1-mutated vs. wild-type patients by using cBioportal. As a result, we obtained a total of 210 DEG's containing 155 upregulated and 55 downregulated genes in RUNX1-mutated patients with a fold change (FC) threshold > 2 and a p-value and q-value < 0.05 (Figure 1 B).
- Next, we selected DEG's s and then performed functional annotation analysis with DAVID separately for up- and down-regulated genes. Interestingly, the KEGG analysis of upregulated genes 10 pathways with a stringent p-value cut off < 0.05, in which the majority of genes are involved in pathways in cancer, focal adhesion, apelin signaling pathway, ABC transporters, small cell lung cancer, JAK-STAT signaling pathway, toxoplasmosis, proteoglycans in cancer, endocrine resistance, hematopoietic cell lineage and amoebiasis (Figure 1 C), (Table S1).
- Next, we focused on KEGG pathway analysis (p-value <0.05) of downregulated genes, from which we obtained only one pathway identifying the genes involved in Staphylococcus au- reus infection (Table S2).



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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 2. (A) Percentage of *RUNX1* mutations in TCGA-AML patients. (B) Volcano plot showing the DEG's between *RUNX1*-mutated vs wild-type patients. (C) KEGG pathway analysis of upregulated genes in *RUNX1*-mutated patients

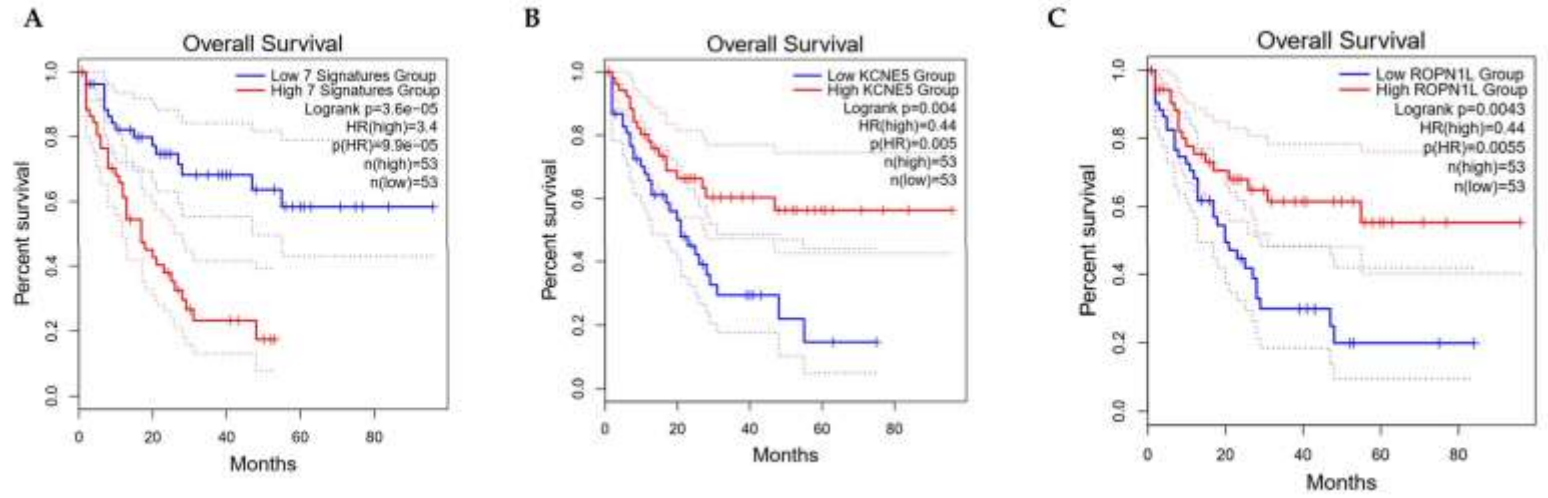

Figure 3. The survival plots showing the overall survival analysis of the seven gene signature (RMAGS) (A) highly expressed in the *RUNX1*-mutated TCGA-AML patients along with the two down regulated genes (B, C)

Table S1 : Functional annotation analysis of upregulated genes associated with *RUNX1* mutations				
KEGG pathways				
Term	Count	%	PValue	Genes
hsa05200:Pathways in cancer	14	9.03226	1.58E-04	NOS2, IL15, LAMA3, IGF2, GNG11, ESR1, GLI3, GNAI1, PTK2, LRP6, AR, CCND1, AKT3, IL12RB2
hsa04510:Focal adhesion	8	5.16129	8.26E-04	CCND1, AKT3, LAMA3, FLNB, DOCK1, PTK2, ITGA9, MYLK
hsa04371:Apelin signaling pathway	6	3.87097	0.0041519	CCND1, NOS2, AKT3, GNG11, GNAI1, MYLK
hsa02010:ABC transporters	4	2.58065	0.0048662	ABCA6, ABCA9, ABCA12, ABCG2
hsa05222:Small cell lung cancer	5	3.22581	0.0053251	CCND1, NOS2, AKT3, LAMA3, PTK2
hsa04630:JAK-STAT signaling pathway	6	3.87097	0.008719	CCND1, IL15, AKT3, AOX1, IFNLR1, IL12RB2
hsa05145:Toxoplasmosis	5	3.22581	0.0105653	NOS2, AKT3, LAMA3, HLA-DOA, GNAI1
hsa05205:Proteoglycans in cancer	6	3.87097	0.0201968	CCND1, AKT3, IGF2, FLNB, ESR1, PTK2
hsa01522:Endocrine resistance	4	2.58065	0.0391938	CCND1, AKT3, ESR1, PTK2
hsa04640:Hematopoietic cell lineage	4	2.58065	0.0402031	CD1E, DNTT, HLA-DOA, CD34
hsa05146:Amoebiasis	4	2.58065	0.0433102	NOS2, LAMA3, CD1E, PTK2

Table S2 : Functional annotation analysis of upregulated genes associated with *RUNX1* mutations				
KEGG pathways				
Term	Count	%	PValue	Genes
hsa05150:Staphylococcus aureus infection	4	7.27273	0.0020342	KRT18, KRT17, C3AR1, DEFB1
hsa04613:Neutrophil extracellular trap formation	3	5.45455	0.0910761	CTSG, AZU1, MPO
hsa05202:Transcriptional misregulation in cancer	3	5.45455	0.0935359	NTRK1, CCNA1, MPO

- Overall, functional annotation analysis showed that the genes that are upregulated in the *RUNX1* mutated patients are involved in a variety of signaling 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 2 A).

- The genes with poor prognosis identified in our study are EGFEM1P, DOCK1, HTR1F, CALCRL, HOPX, TRIM9 and MYLK. These results clearly indicate that increased expression of genes associated with RUNX1 mutations acts as a biomarker in AML patients. We considered these seven genes to be RUNX1 mutations associated gene signatures (RMAGS) in AML. Notably, higher expression of two downregulated genes such as KCNE5, ROPN1L showed a good prognosis in AML patients (Figure 2 B, C).

4. Conclusion

1. Taken together, our results identified a list of genes that are associated with the RUNX1-altereations in AML patients and we named them as RMAGS.

2. Increased expression of RMAGS predicts poor survival in AML patients.

3. These seven genes may act as prognostic biomarkers individully and combindly and can be used to identify the RUNX1 mutation staus in AML patients.

4. 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 tumor growth and drug resistance.

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