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Identification of Differentially Expressed Genes and Key Pathways Associated with PTPN11 Mutation in Acute Myeloid Leukemia

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Abstract: **PTPN11 is a prominent oncogene in many tumors, which linked with poor prognosis. But the gene expression profile associated with this gene mutation in AML is not yet fully elucidated. So, the current study was intended to explore the key genes and pathways associated with AML with PTPN11 mutation. Using the TCGA database, we investigated the gene expression profile for PTPN11 mutation and wild type. EdgeR, an R platform, was applied to categorize the differentially expressed genes. GO and KEGG enrichment analysis was accomplished using DAVID and GSEA. PPI network is investigated with STRING database and Cytoscape software. A statistical investigation was employed to examine the expression levels of genes and prognostic values. As a result, in total 3268 gene s were differentially expressed. GO, KEGG pathway and GSEA analysis of up and down-regulated genes represented that gene were enriched in apoptosis and metabolic signaling pathways. Furthermore, ten hub genes GAPDH, HSP90AA, MYC, UBB, HSPA4, EP300, CCDS, POLR2A, EFTUD2, RPL11. GAPDH was identified from the PPI network. A statistical investigation revealed that PTPN11 gene mutation associated with AML patients had a shorter median survival time with an increased risk of death compared with those without mutations.**

Index Terms: **Acute Myeloid Leukemia, Bioinformatics, Differential expression, Hub genes, PTPN11**

I. INTRODUCTION

Acute Myeloid Leukemia (AML) is a common type of hematological malignancy characterized by uncontrolled proliferation of hematopoietic precursors and loss of the ability to differentiate (Estey, 2016). The estimated overall survival of AML is 8.5 months; the 2 -year and 5-year survival is 32% and 24% (Siegel et al., 2019). Although great advances have been made in the therapeutic methods for AML, still prognosis is far from ideal. However, there are some comprehensive studies, many AML patients with low cure rate are drug resistant and will eventually experience early recurrence. Such recurrence rate is increasing every year, more likely to cause resistance to chemotherapy and failed remission of leukemia stem cells. Furthermore, the incidence of AML had been more and a subset of patients died within one year due to distinct metastasis. Therefore, the molecular association in the occurrence and prognosis of AML needs to be explored, which will contribute to the finding of diagnosis and prognostic markers, and therapeutic targets of AML.

Due to the revolution in techniques and the cost reduction of next - generation sequencing (NGS) of DNA there are several studies which have categorized the AML based on the cytogenetics and molecular mutations (Renneville et al., 2008). Surprisingly, the landscape of mutated genes across

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all studied cases revealed that AML cancers present the lowest mutation level among other adult types of cancer. The average of mutated genes accounts only for 13 mutations per case, of which 5 (*DNMT3A*, *FLT3*, *NPM1*, *IDH1*, *IDH2*, and *CEBPA*) were recurrently mutated, indicating potential targeted therapy (Tomczak et al., 2015).

PTPN11 (Protein Tyrosine Phosphatase Non-Receptor type 11) is a critical component has signa ls for transduction of several growth factor–, hormone-, and cytokine-signaling pathways controlling developmental processes and hematopoiesis as well as energy balance and metabolism (Martelli et al., 2006; Parcells et al., 2006). It is invol ved in enhancing its role in most pathways (Yuan et al., 2020). Among patients with AML treated with high - intensity chemotherapy, those with PTPN11 mutations resulted in the worst RFS and OS (Alfayez et al., 2021). However, the changes in biological processes and signal pathway PTPN11 mutation cause had not been reported. Till to date, an integrated analysis has not yet been performed to gain insight into the impact of mutation on pathways and prognosis of AML patients associated with AML mutation.

Several bioinformatic studies had been reported on various disorders like metastatic uveal melanoma (Xie et al., 2020), myelodysplastic syndrome (Le, 2019), breast cancer (J. L. Deng et al., 2019), hepatocellular carcinoma (Zhang et al., 2021), etc. based on various tools. But these literatures have several limitations: (i) those studies are based on only the sample groups (disease and normal) (ii) and these results are not applied to impact of specific gene mutation on pathways and gene expression. Thus, the identification of potential genes could offer hints for validation and clinical application. Moreover, bioinformatic studies on FLT3 (Chen, Chen, Zhu, et al., 2020), RUNX1 (Zhu et al., 2018), TP53 (Huang et al., 2018), DNMT3A (Chen, Chen, Lu, et al., 2020) mutations have recently revealed several genes and pathways (PI3K-Akt signaling, ECM -receptor interaction pathway etc.), which are helpful in finding out the most significant biomarkers for diagnosis and new therapeutic targets for treatment.

Over the years, bioinformatic study based on gene expression of RNA-seq has seemed as an effective new approach to diagnose the important molecular pathogenesis of tumors. A few publicly accessible databases from large patient associates have been built, which provide the chance to distinguish biomarkers in correlation with disease expansion and treatment response. Signaling pathways such as RTKs (Matthews et al., 1991), MAPK (Platanias, 2003), Ras/RAF/MEK/ERK (Parcells et al., 2006; Platanias, 2003; Shaw & Cantley, 2006) and P13K/AKT pathway (Martelli et al., 2006) were associated in pathogenesis of AML. In any case, a positive and definite outcome regarding PTPN11 mutation in AML is still inadequate. Theref ore, the aim of the present study was to find the key genes and pathways involved in PTPN11 mutations of AML.

In the present study, by means of a series of bioinformatic tools, we acknowledged differentially expressed genes in RNA -seq data with PTPN11 mutation. Then the differentially expressed genes (DEGs) were exposed to pathway enrichment and gene ontology (GO) analysis. A Protein-Protein Interaction (PPI) network and clusters were created, visualized and analyzed, and hub genes were identified. Moreover, we analyzed overall survival of AML patients with PTPN11 mutation. Identification of a characteristic signature of mutant PTPN11 AML may help to determine the chemotherapeutic response to other chemotherapeutic agents for the individualized se lection of therapies and regimens.

II. Materials and methods

A. Data collection

To compare genes and mRNA expression between PTPN11 mutation and wild-type adult AML patients, mRNA expression pattern and corresponding survival profiles were obtained from The Cancer Genome Atlas (TCGA) database.

B. Identification of DEGs

EdgeR (Robinson et al., 2009) was used to screen DEGs between PTPN11 mutation and wild-type AML patients according to the user's guide. DEGs were identified with the cut-off value of log2|fold change (FC)| and P-Value 0.05. A heat map and volcano plot of DEGs were drawn by the ggplot2 package in the R platform.

C. Functional annotation and pathway enrichment analysis

Database for Annotation, Visualization and Integrated Discovery (DAVID) (Jiao et al., 2012)was used to annotate input genes, classify gene functions, identify gene conversions, and carry out Gene Ontology (GO) term analysis. To identify the DEG's functional annotation, we analyzed GO terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment with DAVID, while specifying a P -value < 0.05 for statistical significance.

D. Geneset enrichment analysis

To investigate the effect of PTPN11 mutations on various biological function gene sets in adult AML patients, differences in gene mRNA expression levels of biological functional annotation and pathways between PTPN11 mutation and wil d- type patients were analyzed by GSEA v2-2.2.3 (F. Zhang & Zhong, 2018). Reference gene sets from the Molecular Signatures Database (MSigDB) of c2 (c2.cp.kegg.v5.2.symbols.gmt) and c5 (c5.bp.v5.2.symbols.gmt; c5.mf.v5.2.symbols.gmt; c5.cc.v5.2.symbols.gmt; consist of genes annotated by the same GO terms). The MSigDB of c2 is a pathway gene set, whic h was curated from publications and extracted from canonical pathways and experimental signatures, whereas the MSigDB of c5 was constructed on genes annotated by the same GO terms. The

number of permutations was set at 1,000. Enrichment results satisfying a nominal P-value cutoff of 0.05 with a false discovery rate (FDR),0.25 were considered statistically significant.

E. Integration of PPI network

The Search Tool for the Retrieval of Interacting Genes (STRING) database is an online tool to evaluate prote in-protein interaction information. To evaluate the Protein -Protein interaction relationships among DEGs, we mapped the DEGs to STRING to evaluate the validated interactive relationships among DEGs. Experimentally validated interactions with a combined sco re>0.4 were selected as significant. Using the PPI networks. The cytoHubba plugin in Cytoscape (Paul Shannon et al., 2003) was performed to identify hub genes of the PPI network with defaults. GO enrichment terms of hub genes and genes in modules were also analyzed by Metascape (Zhou et al., 2019).

F. Statistical analysis

All the statistical analyses were conducted with Graph Pad Prism version 9.0 (Swift, 1997). The t-test was used to evaluate the gene expression level between PTPN11 mutation and wild -type AML. The Kaplan-Meier was used to calculate the overall survival of patients Hazard Ratio. P-value <0.05 was indicated as statistically signific

III. RESULTS

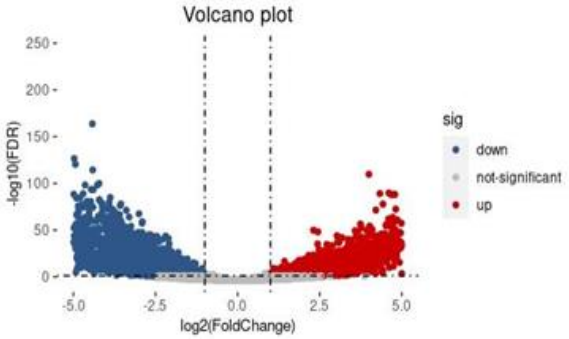
A. Data Source

Information for 170 patients with adult de novo AML and corresponding bone marrow RNA-seq datasets were obtained from the TCGA database. There were 19 AML patients with PTPN11 mutation.

B. Identification of DEGs

To understand the relevant process and pathways affected by PTPN11 mutation we screened the DEGs between PTPN11 mutation and wild-type AML. A total of 3268 DEGs (1427 upregulated and 1841 downregulated) were identified (**Figure. 1**). Top 10 up and down regulated genes were represented (**Table I**).

Fig. 1. Volcano plot of comparing all of the significant DEGs from PTPN11 mutation and PTPN11 wild type samples



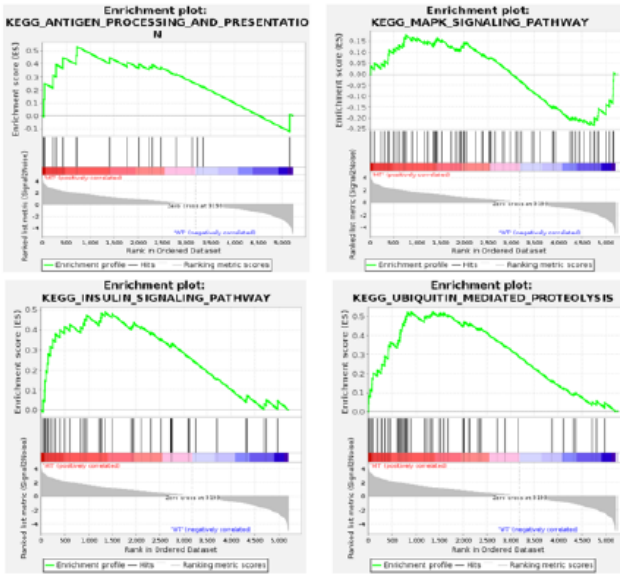
C. GO and KEGG enrichment analysis

In total, 3268 DEGs were submitted for GO and KEGG pathway analyses with DAVID, respectively. For biological processes, DEGs suggested significant enrichment in apoptotic processes. For cellular components and molecular function, D EGs were enriched in the cytoplasm and protein binding (**Table II**). KEGG pathway analysis was also conducted for DEGs. The result showed DEGs were highly enriched in metabolic pathways (**Table III**).

D. GSEA analysis

To investigate the effect of TP53 mutations on the prognosis of AML patients, the effects of PTPN11 mutations on various biological functional gene sets were analyzed by the GSEA approach (**Figure. 2)** In the GSEA analysis of GO enrichment biological processes about regulation of telomeres v ia telomere lighting vesicle budding from membrane, DNA biosynthetic process, positive regulation of DNA metabolism process, telomerorganization were significantly enriched. This suggests that PTPN11 mutations may contribute to disease progression and affect prognosis by influencing cell differentiation, proliferation, and cell adhesion in AML patients. However, the GO enrichment analysis of molecular function was significantly enriched in ubiquitin like protein binding, cadherin binding, mRNA binding, cell adhesion molecular binding. Furthermore, the cellular component was enriched for inner mitochondrial membrane protein, procatalytic splisome, ribonucleic complex, and mitochondrial protein complex. In the GSEA analysis of KEGG pathways, the PTPN11 mutation group was associated with the antigen processing and presentation, MAPK -signalling pathway, Insulin signalling pathway, Ubiguitin mediated proteolysis.

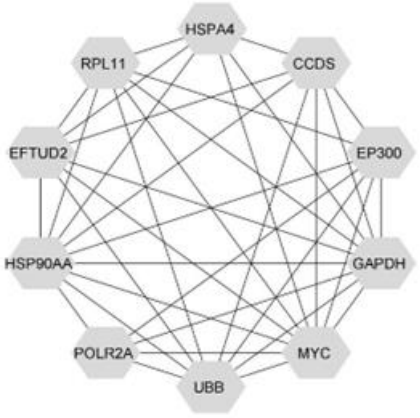
Fig. 2. GSEA results of PTPN11 mutation AML.



E. PPI Network Analysis

We constructed the protein-protein interactome networks and identified some PTPN11 mutation -associated hub genes (**Figure. 3**). The top 10 genes ranked by degree were identified as hub genes, including GAPDH, HSP90AA, MYC, UBB, HSPA4, EP300, CCDS, POLR2A, EFTUD2, RPL11. GAPDH had the highest degree of nodes. GO analysis of Metascape suggests that hub genes are significantly enri ched in metabolic, developmental, and biological processes which have been associated with cancer (**Figure. 4**).

Fig. 3. The PPI network of 10 hub genes with high degree of connectivity



F. Survival analysis

In this study, we observed that PTPN11 gene mRNA expression was dissimilar between PTPN11 mutant and Wild -type patients (**Figure. 5**). Overall survival analysis for AML patients grouped by PTPN11 mutation revealed that AML patients with PTPN11 mutation had a shorter median survival time (MST) than those without PTPN11 mutations. Multivariate Cox proportional hazards regression analysis suggested that PTPN11 mutations were significantly associated with a poor clinical outcome and an increased risk of death, compared to these patients without PTPN11 mutations (**Figure. 6**).

Fig.5. The comparison of mRNA expression between AML patients with PTPN11 mutation and wild type

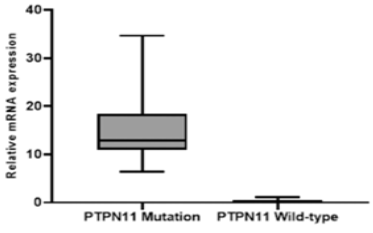
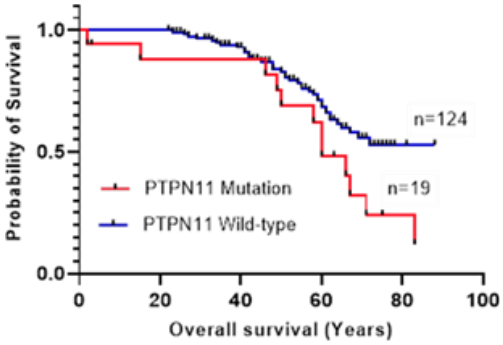


Fig. 6. Kaplan-Meier survival curves for AML patients stratified by PTPN11 mutation



IV. DISCUSSION

AML is characterized by proliferative, clonal, abnormally differentiated, and occasionally poorly differentiated cells of the hematopoietic system, which infiltrate the bone marrow, blood, and other tissues. It is well accepted that genetic variants can be used as independent prognostic biomarkers for AML due to their potential efficacy of chemotherapy. PTPN11 mutations have been reported as a dangerous element in AML. PTPN11 might be a novel biomarker for the early diagnosis and treatment of AML.

Our study showed that PTPN11 expression was lower in wild -type AML compared with PTPN11 mutation. Survival analysis indicated that mutated leukemia patients had shorter overall survival and increased risk of poor clinical outcomes. However, the changes in biological process and signaling pathway PTPN11 mutation cause had not been reported. Herein, we used an RNA - seq dataset of adult AML from the TCGA database to identify the key genes and pathways associated with PTPN11 mutation via bioinformatics analysis. Altogether, 3268 differentially expressed genes were identified, of which 1427 were upregulated and 1841 were downregulated.

GSEA analysis the present study suggested that PTPN11 mutations were significantly associated with the regulation of cell ular metabolism. GO analysis showed that DEGs were notably abundant in apoptosis. It was suggested that PTPN11 mutations may contribute to disease progression and affect prognosis by influencing cell proliferation and hemopoiesis in AML patients. KEGG enri chment analysis revealed that DEGs were enriched in various metabolic process and signaling pathways.

Consistent with previous studies, metabolic pathways have been reported that affect the pathogenesis and prognosis of AML. Furthermore, we built the prote in-protein interactome networks and selected some hub genes with high connectivity involved in PTPN11 mutation AML. The results of GO enrichment analysis of hub genes were similar to previous analysis of DEGs.

Fig. 4. GO term analysis of the top 10 hub genes by Metascape.

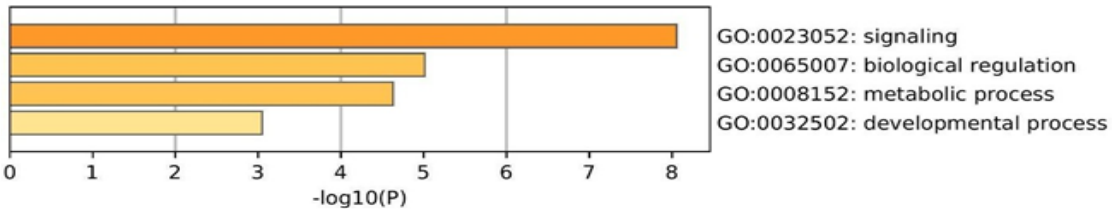


Table I. Top 10 up and down regulated genes between PTPN11 mutation and wild -type

Significance	Gene ID
Up	MT-CO2, RPL4, MT-ND6, TPT1, HNRNPA1, RPL11, HSP90B1, RPL36AL, RPL7, H1 -2
Down	MT-CO2, RPL4, MT-ND6, TPT1, HNRNPA1, RPL11, HSP90B1, RPL36AL, RPL7, H1 -2

Table II. Gene Ontologies of DEGs involved in PTPN11 mutation associated AML

Term	Total Genes	%	P-Value
Biological process			
GO:0006412~translation	71	2.282224	7.30E-07
GO:0000398~mRNA splicing, via spliceosome	59	1.896496	3.99E-05
GO:0006281~DNA repair	61	1.960784	6.10E-05
GO:0098609~cell-cell adhesion	67	2.153648	1.25E-04
GO:0051301~cell division	82	2.635808	1.46E-04
GO:0006364~rRNA processing	54	1.735776	3.58E-04
GO:0015031~protein transport	88	2.828672	4.89E-04
GO:0007067~mitotic nuclear division	54	1.735776	0.010349
GO:0006915~apoptotic process	108	3.471553	0.021551
GO:0007049~cell cycle	46	1.478624	0.027819
GO:0042981~regulation of apoptotic process	44	1.414336	0.045779
Cellular component			
GO:0005737~cytoplasm	1005	32.30473	6.45E-20
GO:0016020~membrane	485	15.58984	3.75E-19
GO:0005654~nucleoplasm	579	18.61138	3.10E-17
GO:0005829~cytosol	664	21.34362	1.34E-15
GO:0005634~nucleus	976	31.37255	1.64E-10
GO:0005739~mitochondrion	281	9.032465	6.67E-09
GO:0070062~extracellular exosome	524	16.84346	2.64E-07
Molecular function			
GO:0005515~protein binding	1713	55.06268	2.68E-41
GO:0044822~poly(A) RNA binding	279	8.968177	2.86E-15
GO:0005524~ATP binding	322	10.35037	1.66E-09

Table III. Enriched pathways of DEGs involved in PTPN11 mutation associated AML

Term	Count	%	P-Value
hsa04141:Protein processing in endoplasmic reticulum	59	1.896496	4.51E-09
hsa00190:Oxidative phosphorylation	39	1.253616	2.15E-04
hsa01200:Carbon metabolism	34	1.092896	3.55E-04
hsa04022:cGMP-PKG signaling pathway	39	1.253616	0.006644
hsa01100:Metabolic pathways	226	7.264545	0.012203
hsa04120:Ubiquitin mediated proteolysis	32	1.028608	0.030481

MYC gene expression is mainly associated with increased risk of AML (Handschuh et al., 2018). Researchers have revealed that GAPDH as a novel clue was involved in tumor progression and serves as a therapeutic target (J. Y. Zhang et al., 2015). Gene expression changes in HS90AA related to chemoresistance (Chu et al., 2013). In addition to that, HSP90AA signaling builds the tumor microenvironment and serves as a therapeutic target (Poggio et al., 2021).

POLR2A encodes the largest subunit of RNA polymerase II complex. Moreover, uncontrolled activation of POLR2A confers an impact on cell survival (Xu et al., 2019). EP300 gene expression plays a dominant role in colorectal cancer (Kowalczyk et al., 2017).

Few studies reported that UBB was highly expressed in NSCLC tissues and several cancers (Deng et al., 2020; Scarpa et al., 2020), in which their expression levels seemed to be essential to sustain the high proliferation rate of cancer cell s and to support their ability to overcome increasing cellular stress. So, the present study reveals that PTPN11 mutation has the significant effect on AML patients.

However, no study has reported the association of HSPA4, CCDS, and EFTUD2 with AML or othe r cancers. Thus, it is important to further explore the role of HSPA4, CCDS, and EFTUD2 in the pathogenesis and prognosis of AML.

CONCLUSION

Our study provides a comprehensive analysis of the prognostic signature of PTPN11 mutation and its underlying gene expression influence profiles in AML. By exploring the TCGA database, we have identified 3268 DEGs that are associated with AML progression and cancer. Our comparative genomic analysis of 143 AML patients indicates that the mutation of the PTPN11 gene is related to poor overall survival in patients. We have also identified 10 potent biomarkers (GAPDH, HSP90AA, MYC, UBB, HSPA4, EP300, CCDS, POLR2A, EFTUD2, RPL11) based on computational screening. The important functions, pathways, and biomarkers which were identified might be helpful for innovation in medical research to easy diagnosis and treatment for target-based therapeutics.

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