In silico analysis of genes and pathways related to acute myeloid leukemia presenting leukopenia

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Abstract

Acute myeloid leukemia (AML) is a type of blood cancers that begins from progenitor and hematopoietic stem cell. Chromosomal abnormalities include balanced translocations between two chromosome like t[8;21] and t[15;17]) in malignancies cells. The present study aimed to explore the AML presenting with leukopenia and gene expression changes induced in High-white count B-cell and Low-white count B-cell the total number of samples is ten. The raw gene expression profiles (ID: GSE20482) of bone marrow achieve from AML patient five High-white count B-cell and five Low-white count B-cell were expressed genes used to recognize differentially. These genes that correspond to official gene symbols were select for protein-protein interaction (PPI) and sub-network construction (score > 0.4). The functional annotation of Gene Ontology (GO) and pathways analysis were performed for those genes involve in networking.

Results

The total number of 846 genes were identified differentially expressed gene and 406 gene were up-regulated another 440 gene were down-regulated. Other 14 genes are interacting with each other significantly identified. Including Hub genes DEGs GNB4, LAMTOR2, ACTN4, HGSNAT, TMED1 are up-regulated while down-regulated DEGs forming hub nodes were UBR4, FBXO30, KLHL21, DCTN6, RNF123, RNF114. AML has a major effect on the expression of genes involved in cell differentiation, apoptosis, cell signaling and modification of protein. AML cells enter the blood quickly and spread to the liver, spleen, and central nervous system. These are total thirteen pathways were enriched and these genes related to oxidative phosphorylation, regulation of actin cytoskeleton, endocytosis, phagocytosis, shigellosis, epithelial cell signaling in helicobacter, adherent junction, pertussis, bile secretion, malaria, African trypanosomiasis were found significantly affected by AML.

Conclusions

Hub genes like GNB4 and UBR4 provide as a novel biomarker in AML.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignant disorder of the hematopoietic stem cells these are characterized by abnormal proliferation of immature cell, blast cells and disable the production of normal blood cells⁴. AML is a genetic disease first established by Janet Rowley who discover the somatic chromosomal abnormalities in leukemic cell of patient sample and they shows the balanced translocations of chromosome (i.e. t[8;21] and t[15;17])⁵. AML affect the people worldwide such as leukemia, lymphoma and multiple myelomas and it is the most common types of leukemia in adults, and lowest survival rate of all different kind of leukemia. AML is an infrequent disease they are highly malignant neoplasms and supervise for a large number of cancer-related deaths. Approximately 25% of all leukemia in adults in the West and constitutes the most frequent form of leukemia. In AML the chromosomal abnormalities are found in t(8;21), t(15;17), inv (16), and 11q23. PML-RARA, RUNX1-RUNX1T1, CBF-MYH11, and MLL-fusion genes are produced by these aberrations. Mutations are
generally occurred in transcription factors, signaling of molecules, tumor suppressor genes, epigenetic regulators, RNA splicing factors, and cohesion complexes, with FLT3, NPM1, and DNMT3A are the most frequently mutated genes in AML.

Cytogenetics provide a powerful diagnostic information and also provide the framework for risk stratification in AML patient and it has a numbers of limitations. Some from of technical failures, like cytogenetics cannot relate the gene fusions, for some example NUP98-NSD1, CBFA2T3-GLIS2, and MNX1-ETV6, which forecast the poor outcome in pediatric patient of AML. AML was initially subdivided on the basis of morphology (French-American-British system), those are helpful for the categorization of pathology. Later some genomics and clinical factors are initiate to coordinate with the reaction of chemotherapy and overall survival. In 2017 the European Leukemia Net (ELN) separate into three prognostic group on the basis of genetic mutation and abnormalities in acute myeloid leukemia these are categorized by favorable, intermediate and adverse.

**Material And Methodology**

2.1. Differentially expressed genes and Microarray data Screening in AML

The raw data procure from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) datasets (http://www.ncbi.nlm.nih.gov/geo/) and gene expression profile datasets obtained from (GENE ID: GSE20482). These samples for this data were hematological stem cells bone marrow and peripheral blood from AML obtained presenting with leukopenia. The datasets derived from a study utilize the GPL6848 Agilent-012391 Whole Human Genome Oligo Microarray G4112A platform. These studies are only genes of high and low blood count B-cell samples analyzed through bioinformatic techniques.

2.2. Preprocessing of data and screening of DEGs in AML

Gene expression datasets obtained from NCBI and further these data were used for pre-processed. The expression values of probes communicate to a particular gene has mean calculated to find the advantage of gene expression and additional including up and down-regulated genes were distinguishing by using BiGGEsTs software analysis. The probe-level ideogram is transformed into gene-level ideogram by using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/). These DEGs pick out, calibrate p values < 0.5 and threshold logFC values are > 0.1 for up and < −0.1 for down regulated genes.

2.3 Principal component analysis and heat map generation in AML

Principal component analysis (PCA) was execute and construct the heat map by using the ClustVis software which is available in online and plotted the PCA graph on DEGs data. ClustVis support
maximum file size is larger so it is not feasible to assemble the PCA plot for all gene expression study.

2.4 PPI network creation and generation of sub-network in AML

DEGs data were upload to the STRING v 10.5 (http://www.string-db.org/). It is an online software dataset for anticipate the functional exchange between the proteins and predict a collaborated outcome for protein-protein interaction among gene position and combined outcome N 0.4 was set as the standard. Prepare for modelling networking and sub-network we used cytoscape v 3.2.1. Cytoscape (http://www.cytoscape.org/) is a bioinformatics software for investigation and visualization of biological networks with high throughput data. The assemble and edge coefficient were taken as standard for network construction.

2.5 Analysis of differentially functional expressed genes in AML

The database for annotation, visualization, and integrated discovery (DAVID, http://david.abcc.ncifcrf.gov/) draft of an action is a database combine a comprising set of functional interpretation and huge genes list. Gene ontology (GO) amplification performed as well as biological, molecular and cellular module were implemented by using DAVID v 6.8 and STRING v 10.5. The basis of hyper-geometric distribution, DAVID clutch the genes with exactly similar function or related as a whole set. DEGs related pathways analysis and completed with the Panther Classification System (http://pantherdb.org/)

2.6 Analysis of Interaction network

Biological General Repository for Interaction Datasets (BioGRID) tools are used to identify (https://wiki.thebiogrid.org/doku.php/ORCS:tools) the possible interconnection for acute myeloid leukemia. BioGRID is curated for biological data base as like as protein-protein interactions, genetical or chemical interactions of acute myeloid leukemia.

2.7 Survival analysis

Overall survival analysis for selected hub genes was performed using GEPIA V2.0. The association between mRNA prognosis and expression of acute myeloid leukemia.

Results

3.1. Differentially expressed genes (DEGs) in AML

The total 846 number of DEGs were allow with their formal gene ideogram as well as up and down-regulated DEGs 406 & 440 their p-value is <.05. (Supplementary file 1). Fourteen genes only were recognized to remarkably interconnect with each other. Mean of AML B cell high and low B cell shown in fig. 1. DEGs were affect based on their standard gene expression value.
3.2 Principal component and hierarchical clustering analysis of DEGs in AML

PCA plot shows a distribute plot with axes communicate to two independent principal components 1 and 2 shows as Fig. 2 A and another shows a Heat-map data matrix in which shade gives a recapitulation of the arithmetic divergence. Heat map was fabricating for differentially expressed genes shows as Fig. 2 B. Conceive of screened DEGs along with assemble of Volcano plot shows in fig. 3 A. MD plot release the up and down regulated genes shows in fig. 3 B. Venn diagram showing the overhang between GO terms down-regulated and up-regulated in the AML shows in fig. 3 C. Total 198 gene were set up the novel up regulated and 235 genes were set up the novel down regulated. DEGs were determined based upon standard gene expression value shows in fig. 3 D.

3.3 Protein-protein interaction meshwork in AML

Protein-protein interaction (PPI) of DEGs shown in fig. 4. Pink and blue circle represents up and down-regulated genes. On the basis of combined score estimated through STRING. The total number of 76 gene pairs (combined score N 0.4) were elevate to link together and constitute one prime network which has 69 nodes and 381 edges. Total five sub-networks will eliminate independently. The up-regulated DEGs and down-regulated DEGs forming hub nodes were GNB4, LAMTOR2, ACTN4, HGSNAT, TMED1 and UBR4, FBXO30, KLHL21, DCTN6, RNF123, RNF114. Clustering coefficient and edge were appropriated as the basis of the selection criteria of hub nodes.

3.4 Construction of Sub-network in AML

Five sub-networks (3 nodes and 3 edges in network1; 2 nodes and 1 edge in network 2 and 3 both) were pulled out from the primary network beside using Cytoscape shown in fig.5. Each and every gene in network A were up regulated and network B are down regulated. Another network C, D and E network line shows the correlation between genes of up and down regulated gene.

3.5 Analysis of functional enrichment in AML

Analysis of functional enrichment was carried out and remarkably improve their processes, functions, and components of DEGs (FDR b 0.05) were record in Tables 1 and 2 results of GO shown in Fig. 6.

3.6 Enrichment of KEGG pathway for DEGs in AML

KEGG pathway enrichment examination was carried out and remarkably up regulated KEGG pathway enrichment for DEGs are African trypanosomiasis, malaria, bile secretion, porphyrin and chlorophyll. Down regulated KEGG pathway enrichment for DEGs is Pertussis, adherent junction, epithelial cell signaling, shigellosis, γ R mediated phagocytosis, endocytosis, regulation of actin cytoskeleton, oxidative phosphorylation, infection of Escherichia coli shown in fig.7.

3.7 Survival analysis in AML
For hub genes like LAMTOR2, KLHL21 and UBR4 overall survival was found to be low for their increased expression shown in fig.8.

**Discussion**

Present study is a little bit effort to understand the molecular mechanism and its complexity involved in the AML patient. In this study we used different bioinformatic tools to analyzed high throughput gene expression datasets which expose to 846 differentially expressed genes (DEGs) were identified. The total number of up-regulated gene were 406 while 440 genes were down-regulated. On the basis of GO cluster analysis major biological processes related to DEGs were homeostasis process, apoptosis and modification of protein. LAMTOR2, ACTN4, HGSNAT, TMED10 are up-regulated and UBR4, FBXO30, KLHL21, DCTN6, RNF123, RNF114 were down-regulated respectively. GNB4 gene is located on chromosome 3q26.33. Guanine nucleotide-binding proteins $\beta$-4, which produce signals to communicate receptors and effector molecules. Which is made up three subunits $\alpha$, $\beta$ and $\gamma$. Subunits of G protein are encoded by mammalian cells$^7$. GNB4 gene mainly encodes through beta subunit. The $\beta$ subunits are dominant regulators and as well as it also regulates signal transduction in various signaling systems. The LAMTOR2 was normally acknowledged in a yeast two-hybrid on a specific binding partner of MEK1 $^8$ which assemble in late endosomes beside the adaptor protein LAMTOR2 (p14)$^9$. MP1 and p14 are nearly identical on structurally and very stable heterodimeric complex. Which is essential for ERK stimulation on endosomes$^{10,11}$. Which depends on gene disruption of p14 and p14/MP1-MEK1 signaling complex modulates the endosomal traffic, EGFR degradation and cellular proliferation$^{12}$. These action are determining for early embryogenesis and throughout tissue homeostasis as released by specific deletion of p14 gene in epidermis$^{13}$. ACTN4 gene is actin cross-linking protein which is encoded by human alpha-actin-4 protein. These are correlated with cell motility, invasion and metastasis in cancer$^{14}$. Excessive expression and massive copy number extension of ACTN4 in different cancer tissue has been also reported and they are associated with the imperfect prognosis in diverse type of cancer$^{15}$. The spectrin genes are superfamily which belongs to the alpha actin and it represents a various group of cytoskeletal proteins. Diverse roles of alpha actin are an actin-binding protein in various types of cell. Gene scramble an isoform of actinin non-muscle, which is condensed in the cytoplasm, and elaborate in metastatic processes. HGSNAT gene encodes acetyl-CoA:alpha-glucosaminide N-acetyltransferase is an enzyme that catalyzes acetylation of the terminal glucosamine residues of sulfate prior to its hydrolysis by alpha-N-acetyl glucosaminidase$^{16}$. HGSNAT is located in the lysosomal membrane and they catalyses a trans membrane acetylation in which the terminal glucosamine residue of heparan sulphate acquires an acetyl group so it converts N-acetylgalcosamine. TMED10 genes are trans-membrane protein, that is able to alteration of distinct proteins of different in segment. TMED10 can form more advance oligomeric assemble in cross linking and depend upon the assemble of mIL-1$\beta$ in THP-1 cells. These cells are containing a unique transmembrane domain, a luminal signal peptide (SS), a Golgi dynamics (GOLD) domain, and a C-terminal tail (CT) facing the cytoplasm.

**Down regulation gene**
Ubiquitin-protein ligase UBR4 enzyme (UBR4) encoded by the UBR4 gene in human and it found in chromosome number one. It encodes the protein appears to component of cytoplasm in cytoskeletal 17. UBR4 gene mark to human papillomavirus. These related pathways are PI3K-Akt signaling pathway and Innate Immune System 18. The function of UBR4 gene calmodulin, ubiquitinase ligase activity, transferase activity, binding of zinc ion and it also monitor the integrin-mediated ubiquitination signaling (https://www.genecards.org/cgi-bin/carddisp.pl?gene=UBR4). Therapeutically consistent function of protein interconnection organized by N-terminal acetylation involve assembly of an E2–E3 ubiquitin-like protein–ligation complex, nucleosome binding by an epigenetic regulator, cytoskeletal organization and integrity of the anaphase-promoting complex 18. F-Box Protein 30 (FBXO30) is a Protein Coding gene and it is a paralogous of the FBXO40. It associated with FBXO30 include Nasopharyngeal Carcinoma disease. The related pathways are MHC Class I mediated antigen presentation and Immune System. FBX030 also regulates the chromosome segmentation of oocyte meiosis. (https://www.genecards.org/cgi-bin/carddisp.pl?gene=FBXO30). The gene family of Kelch-like protein 21 (KLHL) is interchangeable signal transduction mechanism as well as protein degeneration along with ligase and cell-cycle regulation 19. KLHLs attached and stain as particular membrane for degradation as well as significance of sequence dissimilarity in the BTB (Broad Complex, Tramtrack, and BricàBrac) domain 20 and they transpire through E3 ligase complex which KLHLs connect to the Cullin-3 (CUL3) 21. The effect of BACK domains and namesake Kelch motifs distinguish KLHLs from other BTB proteins 22. The Kelch motif connect with actin which recognized the coordination of organelles, plasma membrane and cytoskeleton 23. These genes are adapter for BCR (BTB-CUL3-RBX1). Ubiquitin-protein ligase complex are essential for well-organized chromosome arrangement or cytokinesis. The ligase complex control and restrain the chromosomal passenger complex (CPC) from chromosomes and moderate the ubiquitinated AURKB 24. DCTN6 Dynactin (DCTN) is a diverse subunit protein that drives retrograde transport in cells 25. DCTN6 (Dynactin Subunit 6) is a Protein Coding gene which is RGD (Arg-Gly-Asp) motif in the N-terminal region encoded by DCTN6. Adherent effect of macromolecular proteins such as fibronectin and identical biological function is still not recognized. Some pathways relevant to cell cycle, chromosome separation and transport to the Golgi and consecutive modification. Total 6 subunits of DCTN specify to as dynactin 1 to 6 (DCTN1–6) and these subunits of DCTN are severe to the structural and function of DCTN 26, 27. RNF123 the protein encoded by RNF gene contains a C-terminal ring finger domain, a motif present in a variety of functionally distinct proteins those are involved in protein-protein or protein-DNA interactions. These protein shows the E3 ligase activity regarding the cyclin-dependent kinase inhibitor which is also familiar as p27 or KIP1.

**Conclusion**

Present study it is a miniature attempt to understand the molecular mechanisms and its complexity involved in the AML patient sample. In this study we analyzed high throughput gene expression datasets. Although additional study and experimental authentication are still required to certify the results. The crucial biological processes associated to DEGs, found on GO cluster exploration, were metabolic...
process, signal transduction, apoptosis and protein purifying. DEGs which are extensively initiate the hub nodes are LAMTOR2, KLHL21 and UBR4 respectively. For hub genes like LAMTOR2, KLHL21 and UBR4 overall survival was found to be low for their increased expression. The hallmark of cancer is an irregular cellular metabolism. Besides it promotes the glycolysis, lipid biosynthesis and besides play a crucial role in growth of tumor in AML. Most of the carbon sources synthesize fatty acid it is a form of glucose in mammalian cells and it conduct de novo lipid synthesis and building blocks for tumor cell. Thirteen pathways were enriched and genes related to oxidative phosphorylation, regulation of actin cytoskeleton, endocytosis, phagocytosis, shigellosis, epithelial cell signaling, adherent junction, pertussis, bile secretion, malaria, African trypanosomiasis were found significantly affected by AML.

**Declations**

Competing interests: The authors declare no competing interests.

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**Tables**

**Table 1**: Gene Ontology (GO) analysis of up regulated DEGs.
| Category          | Term                                                                 | PValue      | FDR        |
|------------------|----------------------------------------------------------------------|-------------|------------|
| GOTERM_BP_FAT    | GO:0006796~phosphate-containing compound metabolic process            | 2.98E-06    | 0.007334   |
| GOTERM_BP_FAT    | GO:0006793~phosphorus metabolic process                              | 3.25E-06    | 0.007334   |
| GOTERM_BP_FAT    | GO:0032268~regulation of cellular protein metabolic process           | 5.35E-05    | 0.063377   |
| GOTERM_BP_FAT    | GO:0051246~regulation of protein metabolic process                    | 5.62E-05    | 0.063377   |
| GOTERM_BP_FAT    | GO:0030029~actin filament-based process                              | 1.48E-04    | 0.096366   |
| GOTERM_BP_FAT    | GO:0044403~symbiosis, encompassing mutualism through parasitism       | 1.93E-04    | 0.096366   |
| GOTERM_BP_FAT    | GO:0044419~interspecies interaction between organisms                 | 1.93E-04    | 0.096366   |
| GOTERM_BP_FAT    | GO:0030198~extracellular matrix organization                          | 2.07E-04    | 0.096366   |
| GOTERM_BP_FAT    | GO:0016032~viral process                                              | 2.07E-04    | 0.096366   |
| GOTERM_BP_FAT    | GO:0043062~extracellular structure organization                       | 2.14E-04    | 0.096366   |
| GOTERM_BP_FAT    | GO:0044764~multi-organism cellular process                            | 2.37E-04    | 0.097261   |
| GOTERM_BP_FAT    | GO:0080134~regulation of response to stress                          | 3.18E-04    | 0.118647   |
| GOTERM_BP_FAT    | GO:0035336~long-chain fatty-acyl-CoA metabolic process                | 3.42E-04    | 0.118647   |
| GOTERM_BP_FAT    | GO:0007010~cytoskeleton organization                                  | 3.75E-04    | 0.120822   |
| GOTERM_BP_FAT    | GO:0035337~fatty-acyl-CoA metabolic process                           | 4.85E-04    | 0.142612   |
| GOTERM_BP_FAT    | GO:0030036~actin cytoskeleton organization                            | 5.06E-04    | 0.142612   |
| GOTERM_BP_FAT    | GO:0007015~actin filament organization                               | 6.11E-04    | 0.162057   |
| GOTERM_BP_FAT    | GO:1901135~carbohydrate derivative metabolic process                 | 0.001101    | 0.274455   |
| GOTERM_BP_FAT    | GO:0032956~regulation of actin cytoskeleton organization              | 0.001196    | 0.274455   |
| GOTERM_BP_FAT    | GO:0008610~lipid biosynthetic process                                 | 0.001217    | 0.274455   |
| GOTERM_BP_FAT    | GO:0033036~macromolecule localization                                | 0.001309    | 0.281035   |
| GOTERM_CC_FAT    | GO:0070062~extracellular exosome                                      | 4.89E-12    | 1.23E-09   |
| GOTERM_CC_FAT    | GO:1903561~extracellular vesicle                                     | 6.82E-12    | 1.23E-09   |
| GOTERM_CC_FAT    | GO:0043230~extracellular organelle                                    | 6.98E-12    | 1.23E-09   |
| GOTERM_CC_FAT    | GO:0031988~membrane-bounded vesicle                                  | 1.59E-10    | 2.10E-08   |
| GOTERM_CC_FAT     | GO:0044421~extracellular region part | 1.61E-06  | 1.71E-04 |
|-------------------|-------------------------------------|-----------|----------|
| GOTERM_CC_FAT     | GO:0005925~focal adhesion           | 6.53E-06  | 5.63E-04 |
| GOTERM_CC_FAT     | GO:0005924~cell-substrate adherens junction | 7.45E-06  | 5.63E-04 |
| GOTERM_CC_FAT     | GO:0030055~cell-substrate junction  | 9.21E-06  | 6.09E-04 |
| GOTERM_CC_FAT     | GO:0005912~adherens junction        | 2.85E-05  | 0.001435 |
| GOTERM_CC_FAT     | GO:0000323~lytic vacuole            | 2.98E-05  | 0.001435 |
| GOTERM_CC_FAT     | GO:0005764~lysosome                 | 2.98E-05  | 0.001435 |
| GOTERM_CC_FAT     | GO:0070161~anchoring junction       | 4.61E-05  | 0.002033 |
| GOTERM_CC_FAT     | GO:0005576~extracellular region     | 1.56E-04  | 0.006356 |
| GOTERM_CC_FAT     | GO:0005773~vacuole                  | 2.02E-04  | 0.007627 |
| GOTERM_MF_FAT     | GO:0008092~cytoskeletal protein binding | 8.72E-05  | 0.076204 |
| GOTERM_MF_FAT     | GO:0019899~enzyme binding           | 7.15E-04  | 0.196724 |
| GOTERM_MF_FAT     | GO:0016818~hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides | 0.00115   | 0.196724 |
| GOTERM_MF_FAT     | GO:0016817~hydrolase activity, acting on acid anhydrides | 0.001195  | 0.196724 |
| GOTERM_MF_FAT     | GO:0003924~GTPase activity          | 0.001305  | 0.196724 |
| GOTERM_MF_FAT     | GO:0019901~protein kinase binding   | 0.001807  | 0.196724 |
| GOTERM_MF_FAT     | GO:0017111~nucleoside-triphosphatase activity | 0.001865  | 0.196724 |
| GOTERM_MF_FAT     | GO:0016779~nucleotidyltransferase activity | 0.002001  | 0.196724 |
| GOTERM_MF_FAT     | GO:0016462~pyrophosphatase activity  | 0.002194  | 0.196724 |
| GOTERM_MF_FAT     | GO:0019001~guanyl nucleotide binding | 0.002251  | 0.196724 |
| GOTERM_MF_FAT     | GO:0044325~ion channel binding      | 0.002533  | 0.201247 |
| GOTERM_MF_FAT     | GO:0005525~GTP binding              | 0.002974  | 0.216623 |
| GOTERM_MF_FAT     | GO:0003779~actin binding            | 0.004198  | 0.282254 |
| GOTERM_MF_FAT     | GO:0032561~guanyl ribonucleotide binding | 0.00513   | 0.305882 |
| GOTERM_MF_FAT     | GO:0044769~ATPase activity, coupled to transmembrane movement of ions, rotational mechanism | 0.00527   | 0.305882 |
| GOTERM_MF_FAT     | GO:0032403~protein complex binding  | 0.0056    | 0.305882 |
| GOTERM_MF_FAT     | GO:0051015~actin filament binding   | 0.006499  | 0.328758 |
| GOTERM_MF_FAT         | GO:0019900~kinase binding     | 0.006975 | 0.328758 |
|----------------------|--------------------------------|----------|----------|
| GOTERM_MF_FAT         | GO:0045182~translation regulator activity | 0.007147  | 0.328758 |
| GOTERM_MF_FAT         | GO:0005200~structural constituent of cytoskeleton | 0.008236  | 0.359914 |
| GOTERM_MF_FAT         | GO:0030371~translation repressor activity | 0.009062  | 0.374378 |
| GOTERM_MF_FAT         | GO:0015078~hydrogen ion transmembrane transporter activity | 0.009485  | 0.374378 |
| GOTERM_MF_FAT         | GO:0003684~damaged DNA binding | 0.010045  | 0.374378 |

**Table 2**: Gene Ontology (GO) analysis of down regulated DEGs.
| Category          | Term                                                      | PValue      | FDR          |
|-------------------|-----------------------------------------------------------|-------------|--------------|
| GOTERM_BP_FAT     | GO:0046501~protoporphyrinogen IX metabolic process        | 3.44E-08    | 6.86E-05     |
| GOTERM_BP_FAT     | GO:0006779~porphyrin-containing compound biosynthetic process | 3.87E-08    | 6.86E-05     |
| GOTERM_BP_FAT     | GO:0006778~porphyrin-containing compound metabolic process | 6.72E-08    | 6.86E-05     |
| GOTERM_BP_FAT     | GO:0015669~gas transport                                  | 6.79E-08    | 6.86E-05     |
| GOTERM_BP_FAT     | GO:0033014~tetrapyrrole biosynthetic process              | 1.01E-07    | 8.12E-05     |
| GOTERM_BP_FAT     | GO:0033013~tetrapyrrole metabolic process                 | 3.52E-06    | 0.002366     |
| GOTERM_BP_FAT     | GO:0006783~heme biosynthetic process                      | 4.56E-06    | 0.002633     |
| GOTERM_BP_FAT     | GO:0015671~oxygen transport                              | 9.93E-06    | 0.005014     |
| GOTERM_BP_FAT     | GO:0006782~protoporphyrinogen IX biosynthetic process     | 2.20E-05    | 0.009871     |
| GOTERM_BP_FAT     | GO:0042168~heme metabolic process                        | 3.84E-05    | 0.015509     |
| GOTERM_BP_FAT     | GO:0051188~cofactor biosynthetic process                 | 1.32E-04    | 0.048324     |
| GOTERM_BP_FAT     | GO:0030218~erythrocyte differentiation                    | 1.84E-04    | 0.061892     |
| GOTERM_BP_FAT     | GO:0048534~hematopoietic or lymphoid organ development    | 2.22E-04    | 0.068828     |
| GOTERM_BP_FAT     | GO:0021953~central nervous system neuron differentiation   | 2.96E-04    | 0.085258     |
| GOTERM_BP_FAT     | GO:0034101~erythrocyte homeostasis                       | 3.62E-04    | 0.09735      |
| GOTERM_BP_FAT     | GO:0030097~hemopoiesis                                   | 4.36E-04    | 0.110089     |
| GOTERM_BP_FAT     | GO:002520~immune system development                      | 5.65E-04    | 0.13421      |
| GOTERM_BP_FAT     | GO:0055072~iron ion homeostasis                          | 7.66E-04    | 0.171931     |
| GOTERM_BP_FAT     | GO:0021515~cell differentiation in spinal cord           | 8.13E-04    | 0.172802     |
| GOTERM_BP_FAT     | GO:0046148~pigment biosynthetic process                  | 8.99E-04    | 0.181474     |
| GOTERM_BP_FAT     | GO:0002262~myeloid cell homeostasis                      | 0.001208    | 0.232225     |
| GOTERM_BP_FAT     | GO:0018130~heterocycle biosynthetic process              | 0.001338    | 0.235364     |
| GOTERM_BP_FAT     | GO:0051186~cofactor metabolic process                    | 0.001341    | 0.235364     |
| GOTERM_CC_FAT     | GO:0005833~hemoglobin complex                            | 1.30E-09    | 6.64E-07     |
| GOTERM_CC_FAT     | GO:0014731~spectrin-associated cytoskeleton              | 4.88E-04    | 0.124537     |
| Category      | GO:ID                          | Term                                      | P-value | FDR     |
|---------------|--------------------------------|-------------------------------------------|---------|---------|
| GOTERM_CC_FAT | GO:0030863                     | cortical cytoskeleton                      | 7.42E-04| 0.126063|
| GOTERM_CC_FAT | GO:0044448                     | cell cortex part                          | 0.001803| 0.229872|
| GOTERM_CC_FAT | GO:0044445                     | cytosolic part                            | 0.005077| 0.517882|
| GOTERM_CC_FAT | GO:0008091                     | spectrin                                  | 0.014618| 1       |
| GOTERM_CC_FAT | GO:0005829                     | cytosol                                   | 0.035669| 1       |
| GOTERM_CC_FAT | GO:0005938                     | cell cortex                               | 0.038142| 1       |
| GOTERM_CC_FAT | GO:0072562                     | blood microparticle                       | 0.045891| 1       |
| GOTERM_CC_FAT | GO:0031672                     | A band                                    | 0.04615 | 1       |
| GOTERM_CC_FAT | GO:0030864                     | cortical actin cytoskeleton               | 0.053691| 1       |
| GOTERM_CC_FAT | GO:0099568                     | cytoplasmic region                        | 0.054347| 1       |
| GOTERM_MF_FAT | GO:0005344                     | oxygen transporter activity                | 7.41E-06| 0.006183|
| GOTERM_MF_FAT | GO:0019825                     | oxygen binding                            | 6.57E-04| 0.27416 |
| GOTERM_MF_FAT | GO:0003700                     | transcription factor activity,            | 0.00173 | 0.366809|
|               |                                | sequence-specific DNA binding             |         |         |
| GOTERM_MF_FAT | GO:0001071                     | nucleic acid binding transcription factor | 0.001759| 0.366809|
|               |                                | activity                                  |         |         |
| GOTERM_MF_FAT | GO:0008047                     | enzyme activator activity                 | 0.002244| 0.374321|
| GOTERM_MF_FAT | GO:0020037                     | heme binding                              | 0.002743| 0.381242|
| GOTERM_MF_FAT | GO:0046906                     | tetrapyrrole binding                      | 0.004189| 0.47989 |
| GOTERM_MF_FAT | GO:0015399                     | primary active transmembrane transporter  | 0.005179| 0.47989 |
|               |                                | activity                                  |         |         |
| GOTERM_MF_FAT | GO:0015405                     | P-P-bond-hydrolysis-driven transmembrane | 0.005179| 0.47989 |
|               |                                | transporter activity                       |         |         |
| GOTERM_MF_FAT | GO:0042910                     | xenobiotic transporter activity            | 0.006461| 0.538849|
| GOTERM_MF_FAT | GO:0030506                     | ankyrin binding                           | 0.008435| 0.639508|
| GOTERM_MF_FAT | GO:0042626                     | ATPase activity, coupled to transmembrane | 0.011255| 0.663746|
|               |                                | movement of substances                     |         |         |
| GOTERM_MF_FAT | GO:0016820                     | hydrolase activity, acting on acid        | 0.012306| 0.663746|
|               |                                | anhydrides, catalyzing transmembrane      |         |         |
|               |                                | movement of substances                     |         |         |
| GOTERM_MF_FAT | GO:0022804                     | active transmembrane transporter activity  | 0.012817| 0.663746|
| GOTERM_MF_FAT | GO:0003810                     | protein-glutamine gamma-glutamyltransferase| 0.014862| 0.663746|
| GOTERM_MF_FAT | GO:0097159~organic cyclic compound binding | 0.015401 | 0.663746 |
|---------------|---------------------------------------------|-----------|-----------|
| GOTERM_MF_FAT | GO:0051537~2 iron, 2 sulfur cluster binding | 0.015733 | 0.663746 |
| GOTERM_MF_FAT | GO:0044212~transcription regulatory region DNA binding | 0.01618 | 0.663746 |
| GOTERM_MF_FAT | GO:0000975~regulatory region DNA binding | 0.016386 | 0.663746 |
| GOTERM_MF_FAT | GO:0019209~kinase activator activity | 0.016927 | 0.663746 |
| GOTERM_MF_FAT | GO:0001067~regulatory region nucleic acid binding | 0.017304 | 0.663746 |

**Figures**
Figure 1

A. Mean of AML high and low B cell.
Figure 2

A. Principle component 1st and 2nd analysis of database. B. Differentially expresses gene sets of heat map, blue to orange gradation represents the small to large gene expression values change.
3. A. Visualization of screened DEGs through constructed Volcano plot.

B. MD plot reveals the up and down regulated genes (Figure 3A and 3B).

C. Venn diagram showing the overlap between GO terms down-regulated in AML and up-regulated in the AML.

D. 198 gene were found in novel up regulated and 235 gene were found in novel down regulated. DEGs were determined based upon average gene expression value.

Figure 3

A. Visualization of screened DEGs through constructed Volcano plot. B. MD plot reveals the up and down regulated genes (Figure 3A and 3B). C. Venn diagram showing the overlap between GO terms down-regulated in AML and up-regulated in the AML. D. 198 gene were found in novel up regulated and 235 gene were found in novel down regulated. DEGs were determined based upon average gene expression value.
Figure 4

A. Protein-protein interaction (PPI) of differentially expressed genes. Pink circle represents the up-regulated genes and blue circle represent down-regulated genes. B. Protein-protein interaction (PPI) of differentially expressed genes. Pink circle represents the up-regulated genes and blue circle represent down-regulated genes, Pink diamond and Blue diamond similar related genes. Still Gray line shows the correlation between genes and their thickness of lines (edges) is proportional to the combined score.
These five (A, B, C, D, E) sub-networks of differentially expressed genes (DEGs). Pink circle represents the up-regulated genes and blue circle represent the down-regulated genes and lines shows the correlation between these genes.
6. A. Analysis of gene ontology for differentially expressed up regulated genes in AML. B. Analysis of gene ontology for differentially expressed down regulated genes in AML.

**Figure 6**

A. Analysis of gene ontology for differentially expressed up regulated genes in AML. B. Analysis of gene ontology for differentially expressed down regulated genes in AML.
7. **KEGG pathway enrichment for DEGs**

![KEGG pathway enrichment for DEGs](image)

**Figure 7**

KEGG pathway enrichment for DEGs
Figure 8

The correlation between mRNA expression and prognosis of LAMTOR2, KLHL21, UBR4, TMED1, RNF114, ACTN4, in AML.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementryfile1.xlsx