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Original Article

Transcriptome Analysis of Beta-Catenin-Related Genes in CD34+ Haematopoietic Stem and Progenitor Cells from Patients with AML

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Abstract. ***Background*: Acute myeloid leukaemia (AML) is a disease of the haematopoietic stem cells(HSCs) that is characterised by the uncontrolled proliferation and impaired differentiation of normal haematopoietic stem/progenitor cells. Several pathways that control the proliferation and differentiation of HSCs are impaired in AML. Activation of the Wnt/beta-catenin signalling pathway has been shown in AML and beta-catenin, which is thought to be the key element of this pathway, has been frequently highlighted. The present study was designed to determine beta-catenin expression levels and beta-catenin-related genes in AML.**

***Methods*: In this study, beta-catenin gene expression levels were determined in 19 AML patients and 3 controls by qRT-PCR. Transcriptome analysis was performed on AML grouped according to beta-catenin expression levels. Differentially expressed genes(DEGs) were investigated in detail using the Database for Annotation Visualisation and Integrated Discovery(DAVID), Gene Ontology(GO), Kyoto Encyclopedia of Genes and Genomes(KEGG), STRING online tools.**

***Results*: The transcriptome profiles of our AML samples showed different molecular signature profiles according to their beta-catenin levels(high-low). A total of 20 genes have been identified as hub genes. Among these, *TTK, HJURP, KIF14, BTF3, RPL17* and *RSL1D1* were found to be associated with beta-catenin and poor survival in AML. Furthermore, for the first time in our study, the *ELOV6* gene, which is the most highly up-regulated gene in human AML samples, was correlated with a poor prognosis via high beta-catenin levels.**

***Conclusion*: It is suggested that the identification of beta-catenin-related gene profiles in AML may help to select new therapeutic targets for the treatment of AML.**

Keywords: AML; CD34+ cells; Beta-catenin; Microarray; DEGs.

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Introduction. Acute Myeloid Leukemia (AML) is a hematopoietic stem cell (HSC) disorder. It is characterized by the transformation of myeloid progenitor cells, which normally differentiate into mature blood cells during hematopoiesis, into clonally expanding neoplastic cells, resulting in a loss of their differentiation capacity.[1] Normal stem cells, which possess the ability to self-renew, can undergo neoplastic transformation due to mutations in genes associated with signaling pathways, as well as epigenetic or gene expression changes. In this regard, the Wnt signaling pathway, which is active especially in the embryonic period and in various cancer types, has been extensively reported in the literature in recent years.[2-4] The Wnt signaling pathways are classified as canonical (beta-catenin dependent) or non-canonical (beta-catenin independent).[5] The beta-catenin protein undergoes hypophosphorylation and stabilization as a result of canonical Wnt pathway activation, which is crucial for stem cell maintenance. The CTNNB1 gene encodes the protein beta-catenin, which has multiple functions linked to clonogenic growth of cells, differentiation and apoptosis both in vitro and in vivo. This protein is stabilized in the cytoplasm and transported into the nucleus under the control of the canonical Wnt pathway.[5]

In the absence of a Wnt ligand, the main mediator beta-catenin, is constitutively phosphorylated by a destruction complex made up of *GSK3β, CK1*, Axin, and *APC*, making it a target for degradation by the proteasome. When Wnt ligands bind to Frizzled and *LRP5/6*, which are Wnt receptors, the destruction complex becomes saturated with phosphorylated beta-catenin (which cannot be destroyed), causing cytosolic accumulation of non-phosphorylated beta-catenin.[6] From there, beta-catenin ultimately translocates into the nucleus, it displaces Groucho and binds to *TCF/LEF*, resulting in the activation of proto-oncogenic Wnt target genes.[7]

Numerous studies on the gene expression levels of beta-catenin, an essential component of the Wnt pathway, have been reported in the literature. Serinsöz et al. (2004),[8] Simon et al. (2005),[9] Ysebaert et al. (2006),[10] Xu et al. (2008),[11] Chen et al. (2009),[12] Gandillet et al. (2011),[13] Li et al. (2018),[5] Jiang et al. (2018),[14] Morgan et al. (2019),[6] Wagstaff et al. (2022),[7] and Han et al. (2022)[15] are among the research that have made major contributions. These studies, conducted in a consistent manner, have revealed significant differences in beta-catenin expression levels among AML patient groups.

In this study, the main objective was to investigate the gene expression profiles of beta-catenin in patients with AML and to determine the status of beta-catenin levels among AML patient groups. Our data showed that the transcriptome profiles of AML samples, which were categorized into two groups based on the expression levels of the beta-catenin gene, were different from the transcriptome profiles of healthy control samples. The differentially expressed gene sets were then examined by cluster analysis. It has been observed that a significant signature is obtained from the comparison of transcriptome profiles between AML patient groups with high and low levels of beta-catenin. Furthermore, the biological functions, altered signaling pathways, and protein-protein interaction network of these DEGs were analyzed and discussed. This study is the first global transcriptomic investigation conducted in AML based on the levels of beta-catenin, the key component of the Wnt signaling pathway. In our study, the identification of beta-catenin associated genes and pathways that play a crucial role in cancer development can provide important insights into the molecular biology of AML. In addition, novel diagnostic, prognostic and therapeutic candidates may be discovered.

Materials and Methods.

Sample Collection. Blood or bone marrow samples of a total of 19 newly-diagnosed (7 female and 12 male) AML patients and 3 healthy bone marrow donors, who have not been previously diagnosed with any malignant disease, in the Department of Hematology, Faculty of Medicine, Ankara, Gazi and Hacettepe University was included in this study. The median age was 54 years and the median white blood cell count was 48 689. The patients were diagnosed according to the FAB (French-American-British) classification criteria. Ankara University Faculty of Medicine ethics committee (reference number and date: 123-3355 and 07.01.2008) approved this study and written informed consents were obtained from them or their relatives.

Isolation of CD34+ Cells from the Bone Marrow or Blood of AML Patients and Controls. Mononuclear cells were purified from the blood or bone marrow by density gradient centrifugation using Ficoll-Paque (PAA Laboratories, Austria). After centrifugation (1200g, 20 min, 20^0C), mononuclear cells obtained from the interface were washed with phosphate-buffered saline (PBS, Sigma). The CD34+ cells were isolated from the mononuclear cells using positive immunomagnetic selection (EasySep CD34 positive selection kit, Stem

cell Technologies, Canada) according to the manufacturer's protocol. Isolated CD34+ cells were cultured for 2 days at 37°C and 5% CO_2 using Stemspan H3000 (Stem Cell Technologies, Canada), a special medium for hematopoietic stem cells and Stemspan CC100 (Stem Cell Technologies, Canada) cytokine mixture. 4-5x10^5 cells/ml were obtained. The purity of cultured CD34+ cells from AML patients and healthy controls was determined by flow cytometry using $CD34._{PerCP}$, $CD45._{FITC}$, $CD33._{PE}$ (BD Biosciences, San Jose, CA, USA) and was greater than 97% in samples used for microarray analysis.

RNA İsolation. Total RNA was isolated from CD34+ cells using Trizol reagent according to the manufacturer's protocol (Invitrogen, ABD). Total RNA quality and quantity were evaluated using NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer RNA 6000 (Agilent, Technologies, Santa Clara, CA) and agarose gel electrophoresis.

Real-Time Quantitative PCR. Beta-catenin expression was analyzed in AML patients compared to controls using the real-time quantitative PCR method. 1µg of total RNA was reverse-transcribed using random hexamers with Roche Transcriptor High Fidelity c-DNA synthesis kit according to the manufacturer's protocol. Real-time amplification was performed with Light Cycler 480 SYBR Green Master Mix (Roche Applied Sciences, Manheim, Germany) performed on Light Cycler 480 Instrument (Roche Applied Sciences, Manheim, Germany). For normalization hypoxanthine phosphoribosyltransferase (hprt) endogenous control was selected. Beta-catenin and hprt primers designed using the Perl Primer software, were as follows: beta-catenin forward primer:5'-ATTACAACTCTCCACAACCT, beta-catenin reverse primer: 5'- CAGACAGATAGCACCTTCAG and *hprt* forward primer: 5'- TGACACTGGCAAAACAATGCA, *hprt* reverse primer: 5'-GGTCCTTTTCACCAGCAAGCT. Relative beta-catenin expression levels were calculated using $2^{-\Delta\Delta Ct}$ method.[16]

Gene Expression Profiling. 19 AML and 3 healthy control samples were analyzed by microarray analysis. 500 ng of total RNA isolated from each sample was used to generate amplified and biotinylated cRNA with the GeneChip® 3′ IVT Express Kit (Affymetrix, Santa Clara, CA). 15 µg of fragmented biotin-labeled cRNA was hybridized with Human Genome U133 Plus 2.0 Affymetrix GeneChips (Affymetrix, Santa Clara, CA, USA) for 18 hours at 45^0C. Arrays were washed, stained and finally scanned using the GeneChip® Scanner 3000 according to the Affymetrix procedure.

Microarray Data Analysis. AffymetrixGeneChip Command Console software was used to preprocess all of the raw data (CEL files) (version 3.0.1). The expression algorithm used by Affymetrix MAS 5.0 to create the % present calls. Partek Genomic Suite 6.6 software (Partek Inc., St. Louis, MO, USA) was used for analyzing gene expression data. Briefly, robust multiarray averaging (RMA) was used to import the data and normalize it. Filtering the list of transcripts that were differentially expressed was done using a fold change threshold of >1.5 and $p < 0.05$ limits. Optimized false discovery rate (FDR) was used to calculate adjusted P values, often known as q values.[17] The FDR cutoff was q < 0:05, which was seen as significant. The relationships between the AML and control groups were evaluated using unsupervised hierarchical clustering. The microarray data analyses are summarized in **Supp. Figure 1**. Microarray data are available at http://www.ncbi.nlm.nih.gov/geo/ (accession no. GSE245305).

Gene Ontology and Pathway Enrichment Analyses. The Database for Annotation Visualization and Integrated Discovery (DAVID) (version 6.8) (https://david.ncifcrf.gov/) was used to analyze Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. A statistically significant difference was defined as $p<0.05$.

Protein-Protein İnteraction (PPI) Network. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database v10.0 (http://www.stringdb.org/) is an online tool created to analyze the information on protein-protein interaction (PPI).[18] AML-related DEGs were mapped to STRING to evaluate the PPI data; a confidence score of >0.9 was used as the cutoff value, and Cytoscape software (version 3.8.2, www.cytoscape.org) was used to display the results. Statistical significance was defined as a P value less than 0.05. In addition, modules of the PPI network were screened in Cytoscape 3.6.1 using Network Analyzer and the Molecular Complex Detection (MCODE) plugin (degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. depth =100). To identify potential "hub" proteins in Cytoscape, betweenness centrality degrees were calculated.[19]

Survival Analysis of Hub Genes. The median relative expression of genes in the Gene Expression Profile Interactive Analysis (GEPIA) database was used as a cut-off value for determining the prognostic significance of hub genes in AML. The Log Rank test was used to plot and analyze the survival curves for crucial beta-catenin-related genes in AML. The hazard ratio (HR) and 95% confidence interval (CI) were plotted on the survival

curve. It was determined to be statistically significant when $p < 0.05$.

GEPIA is an online tool that was used to validate the relative expression of selected center genes. Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression, GEPIA (http://gepia.cancer-pku.cn/) that provide data on gene expression and survival, are frequently used to evaluate gene expression between cancer and normal samples.[20]

Validation of Microarray Data. DEG lists were used to select genes for microarray validation. Real-time quantitative PCR was performed to validate microarray gene expression data. Relative gene expression of *Grb-2*, *Nf-kb*, *Gsk3ß* genes between AML and control groups were calculated using $2^{-\Delta\Delta Ct}$ method. The expression levels of target genes have been normalized to the *hprt* endogenous control. The ratio of AML/control groups was calculated as the fold-change in expression.

Statistical Data Analysis. The Student's t test and one-way ANOVA were both used for group comparisons, with the Tukey test for pairwise comparisons used for statistically significant ANOVA p values. SPSS statistics version 20 (IBM SPSS Statistics, Chicago, Illinois) was used for all statistical analyses. $p < 0.05$ was considered to be statistically significant. We identified differentially expressed genes concerning the 1.5-fold change threshold. Mann–Whitney U test was applied to compare the qPCR gene expression values between AML patients and control groups. P values are adjusted using the Benjamini- Hochberg procedure. False discovery rates (FDR) less than 10% were considered as statistically significant. Statistical significance was set at $p<0.05$.

Results.

Isolation and Characterization of CD34+ Cells of AML Patients and Controls. AML patients' and healthy control samples' primary cultivated CD34+ cells preserved the CD34+ phenotype at a rate of 98% after culture, and all patient and healthy controls that maintained the CD34+ phenotype were subjected to microarray analysis (**Supp. Figure 2**).

Beta-Catenin Expression Levels in AML Patients and Controls. Beta-catenin expression profiles were analyzed prior to microarray analysis in 19 AML patients compared to controls. 8 AML patients had high beta-catenin gene expression levels (Group 1), while 7 AML patients had low beta-catenin gene expression levels (Group 2). In 4 AML patients, there was no significant increase or decrease ($p<0.05$, **Figure 1**).

The study was completed on two groups:

Group 1; AML patient group with high beta-catenin levels

Group 2; AML patient group with low beta-catenin levels

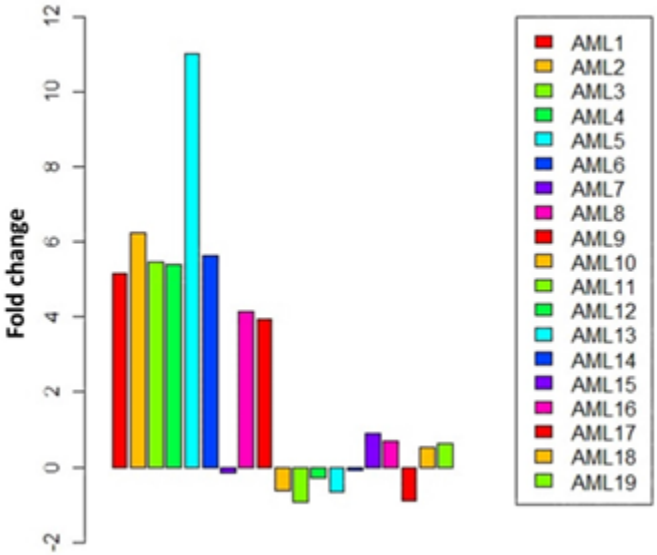

Figure 1. Beta-catenin gene expression levels of AML patients.

Correlation Between Beta-Catenin Expression and Common Clinical Features in Patients. The expression levels of the beta-catenin gene in AML patients did not correlate with the clinical characteristics of the patients ($p>005$) (**Supp. Table 1**).

Identification of Differentially Expressed Genes. According to the venn diagram, when AML patients with high beta-catenin levels were compared to healthy controls (high vs control group), 2889 genes (1619 up- and 1270 down-regulated genes) were differentially expressed (DEGs), whereas 904 genes (576 up- and 328 down-regulated genes) were differentially expressed when AML patients with low beta-catenin levels were compared to controls (low vs control group) after the microarray analysis (**Figure 2**).

In order to identify beta-catenin related genes in the AML group, we focused on genes other than DEGs that were common in the venn diagrams of two distinct groups, high vs control and low vs control. These uncommon genes were the target of all analyses.

Independent of the common gene cluster, we chose 2314 DEGs (1194 up- and 1120 down-regulated genes) in the AML group with high beta-catenin levels (high vs control). Similar to this, only 329 DEGs (151 up- and 178 down-regulated genes) were differentially expressed in the AML group with low beta-catenin levels (low vs cont) (**Figure 2**).

The transcriptome profiles of the AML samples showed different molecular signature profiles according on their beta-catenin levels (high-low) when compared

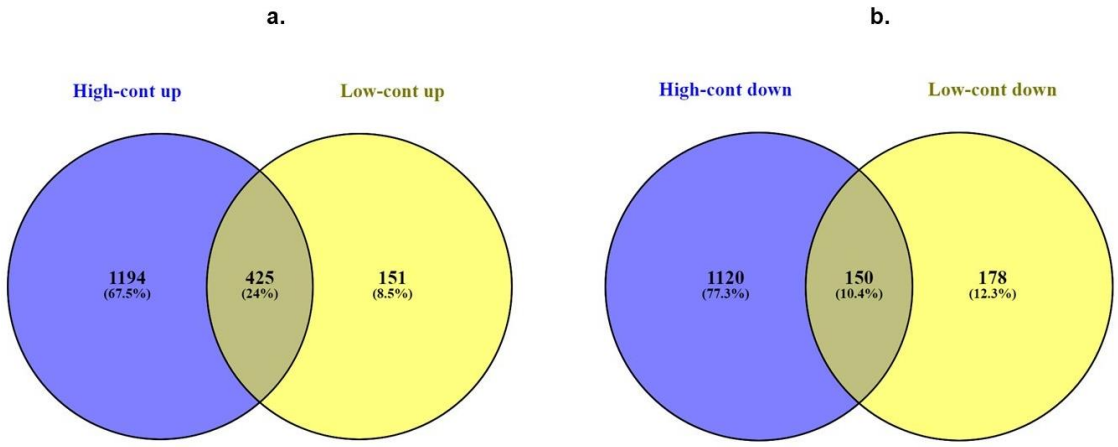

Figure 2. Venn diagram analysis of DEGs. The blue circle represents the DEGs in high vs control group, the yellow circle represents the DEGs in low vs control group. The intersection of the two circles represents overlapping DEGs among the two groups. **a.** including up-regulated genes, **b.** including down-regulated genes, DEGs, differentially expressed genes.

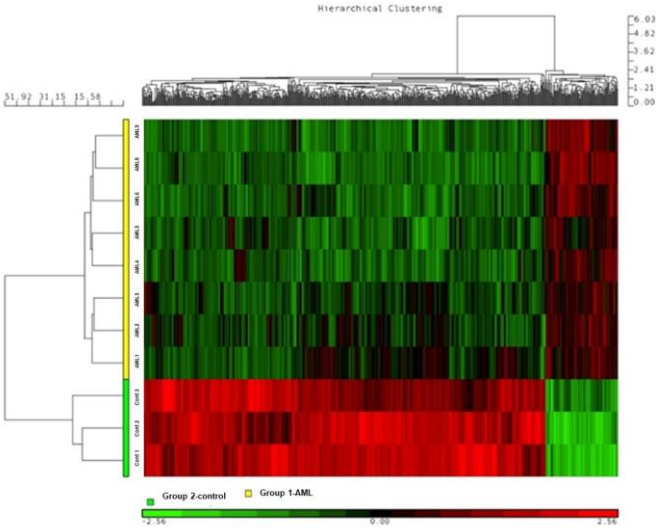

Figure 3. Heat map of hierarchical cluster analysis for AML patient group with high beta-catenin levels compared to controls. Group 1 samples belong to AML patients; Group 2 samples belong to controls. Red color represents highly expressed genes; green color represents low expressed genes.

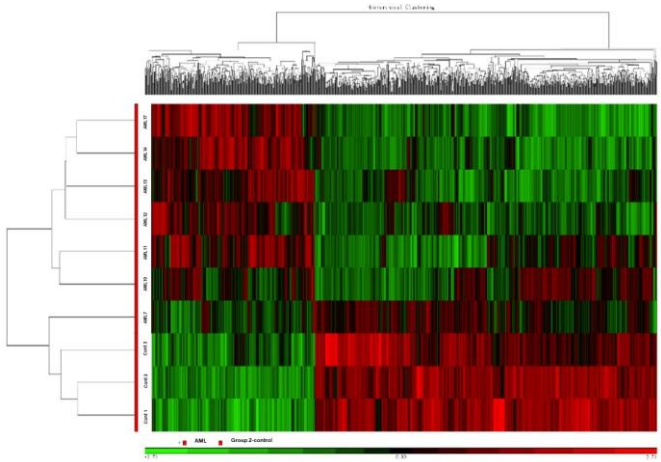

Figure 4: Heat map of hierarchical cluster analysis for AML patient group with low beta-catenin levels compared to controls. Group 1 samples belong to patients; Group 2 samples belong to controls. Red color represents highly expressed genes; green color represents low expressed genes.

to the healthy control group. **Figure 3** and **4** show heat maps for DEGs between the two groups.

The DEGs that are top upregulated and downregulated among the 2 groups are listed in **Table 1**.

Gene Ontology, DAVID and KEGG Pathways Analysis. GO, DAVID, and KEGG pathway analysis software were used to complete functional and pathway enrichment analyses of genes with increasing or reducing expression in order to better understand the function and mechanism of DEGs identified through microarray data analysis. The groups were compared with the control groups.

Comparison-High vs Control Groups. The results of the GO analysis (including Molecular Function, Biological Process and Cellular Component) indicated changes in the biological processes of up-regulated DEGs that were mainly enriched for ATP binding, cell division, protein phosphorylation, cell-cell adherens junction, m-RNA splicing, DNA repair, and beta-catenin -TCF complex assembly. Furthermore, enrichment of down-regulated DEGs was mostly in molecular function and biological processes, including metal ion binding, regulation transcription, ATP binding, protein ubiquitination, cell cycle and protein dephosphorylation (**Figure 5**).

To learn more about biological pathway alterations in the AML group with high beta-catenin levels, the KEGG pathway was studied, and it was discovered that many DEGs in the KEGG pathway were significantly enriched. Significant pathway enrichment results are listed in **Table 2**. As indicated in **Table 2**, the major pathways were protein processing in endoplasmic reticulum, proteoglycans in cancer, Rap1 signaling pathway, cellular senescence, autophagy, pathways in cancer, endocytosis, ubiquitin mediated proteolysis, transcriptional misregulation in cancer, apoptosis and necroptosis pathways.

High vs Control-Upregulated genes			High vs Control-Downregulated genes		
Gene symbol	Fold change	P value	Gene symbol	Fold change	P value
DDX17	9.959	0.0002545	ZMAT3	-8.658	0.0030320
NUSAP1	9.873	0.0195311	MANEAL	-8.159	0.0109299
MAP3K13	9.855	0.0469811	GIMAP6	-7.991	0.0139186
IL1RN	9.824	0.0017295	GPATCH2L	-7.775	0.0067863
HBD	9.783	0.0066013	SHANK3	-7.384	0.0298674
GOLIM4	9.734	0.0002690	PGK1	-7.023	0.0038004
CMTM5	9.671	0.0001062	CHURC1	-6.460	0.0147577
ELOVL6	9.642	0.0011015	MALAT1	-6.390	0.0055627
C5orf20 /// TIFAB	9.465	1,99E+00	FLT3	-6.115	0.0036779
DEFA4	9.448	0.0004017	ADAM10	-5.975	0.0075925

Low vs Control-Upregulated genes			Low vs Control-Downregulated genes		
Gene symbol	Fold change	P value	Gene symbol	Fold change	P value
TMEM246	9.641	8,80E+00	PTPN14	-7.544	0.0328966
ANK1	9.364	0.0002408	ENO1	-7.242	0.0160091
ARHGAP6	9.223	0.0001952	KCNK17	-5.381	0.0485642
CMTM5	8.928	0.0001605	P4HB	-4.693	0.0085150
HBBP1	8.744	0.0030283	KANSL1-AS1	-4.325	0.0332731
LIF	8.589	0.0026764	HINT3	-4.309	0.0072134
BACE2	8.122	0.0113741	DISC1	-4.144	0.0138568
PBX1	7.866	0.0053022	MIR146A	-3.831	0.0043779
PLOD2	7.590	0.0028327	ACTB	-3.817	0.0062677
LDLRAD3	7.584,81	0.0009274	H2BFS	-3.639	0.0268501

Table 1. The top 10 upregulated and downregulated DEGs in high vs control and low vs control (P value <0.05 and |Log2Foldchange| ≥1.5).

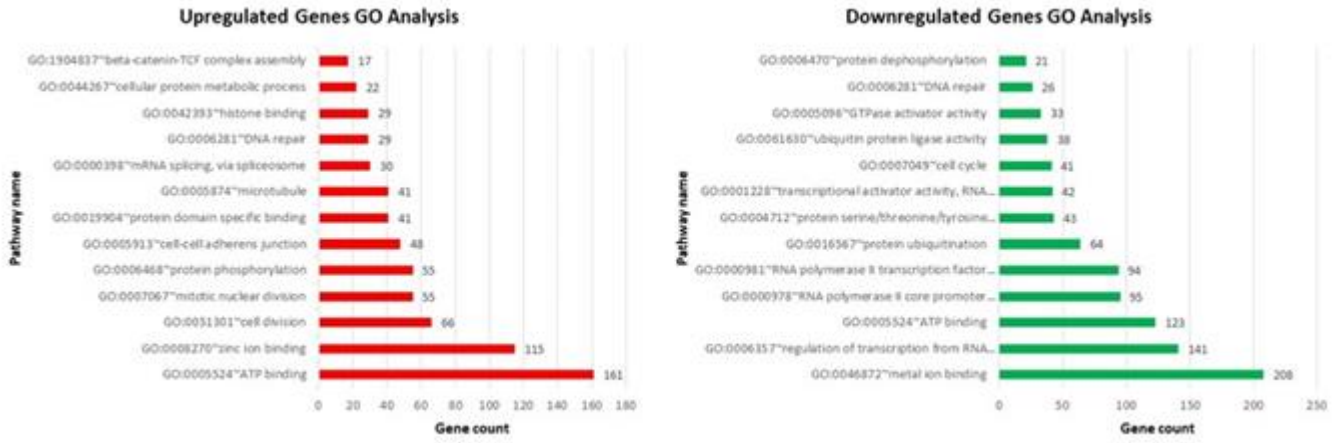

Figure 5. GO enrichment analysis of up-regulated and down-regulated genes in AML patient group with high vs controls.

Table 2: Dysregulated pathways using the KEGG database in AML patient group with high beta-catenin levels vs controls (P value <0.05).

Expression	Pathway ID	Name	Gene count	p value
Up-DEGs	hsa04141	Protein processing in endoplasmic reticulum	37	8,91E+07
	hsa05205	Proteoglycans in cancer	29	0.004
	hsa04015	Rap1 signaling pathway	26	0.036
	hsa04218	Cellular senescence	26	8,26E+11
	hsa04110	Cell cycle	23	5,11E+11
Down-DEGs	hsa04140	Autophagy	23	0.002
	hsa05200	Pathways in cancer	47	0.009
	hsa04144	Endocytosis	34	2,16E+11
	hsa04120	Ubiquitin mediated proteolysis	24	1,42E+11
	hsa05202	Transcriptional misregulation in cancer	23	0.003
	hsa04217	Necroptosis	20	0.003
	hsa04210	Apoptosis	19	0.001
	hsa04218	Cellular senescence	18	0.014

Comparison-Low vs Control Groups. For biological processes, the up-regulated DEGs were mainly enriched in nucleus, nucleoplasm, ATP binding, cell division and DNA repair, whereas the down-regulated DEGs were significantly enriched in regulation of transcription from RNA polymerase II promoter, integral component of plasma membrane, cell adhesion, protein phosphorylation, protein serine/threonine/tyrosine kinase activity, microtubule and cell cycle. **Figure 6** shows all of the detailed GO term enrichment analysis findings.

For overall DEGs, a KEGG pathway analysis was also performed in the AML group with low beta-catenin levels. Significant pathway enrichment results are listed in **Table 3**. According to the results, DEGs were obviously enriched in hematopoietic cell lineage, signaling pathways regulating pluripotency of stem cells, ECM-receptor interaction, ferroptosis, pathways in cancer, regulation of actin cytoskeleton, MAPK signaling pathway (**Table 3**).

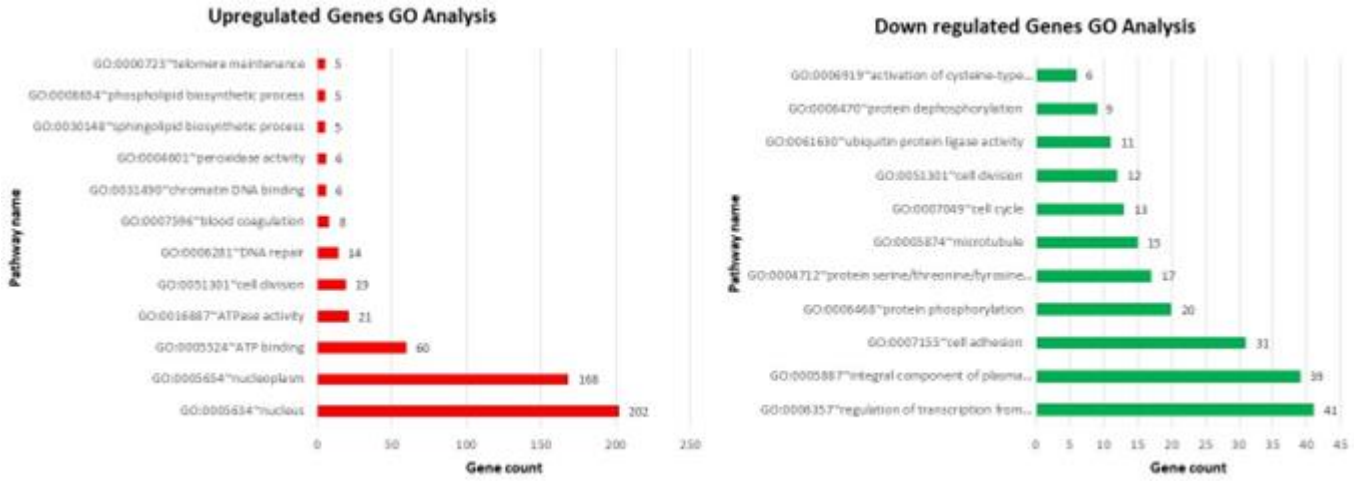

Figure 6. GO enrichment analysis of up-regulated and down-regulated genes in AML patient group with low vs controls.

Table 3. Dysregulated pathways using the KEGG database in AML patient group with low beta-catenin levels vs controls (P value <0.05)

Expression	Pathway ID	Name	Gene count	p value
Up-DEGs	hsa04640	Hematopoietic cell lineage	11	0.001
	hsa04550	Signaling pathways regulating pluripotency of stem cells	11	0.018
	hsa04512	ECM-receptor interaction	8	0.02
	hsa04216	Ferroptosis	6	0.010
Down-DEGs	hsa05200	Pathways in cancer	19	0.006
	hsa04810	Regulation of actin cytoskeleton	11	0.005
	hsa04010	MAPK signaling pathway	11	0.038
	hsa04218	Cellular senescence	9	0.006
	hsa04068	FoxO signaling pathway	8	0.009
	hsa04550	Signaling pathways regulating pluripotency of stem cells	7	0.043

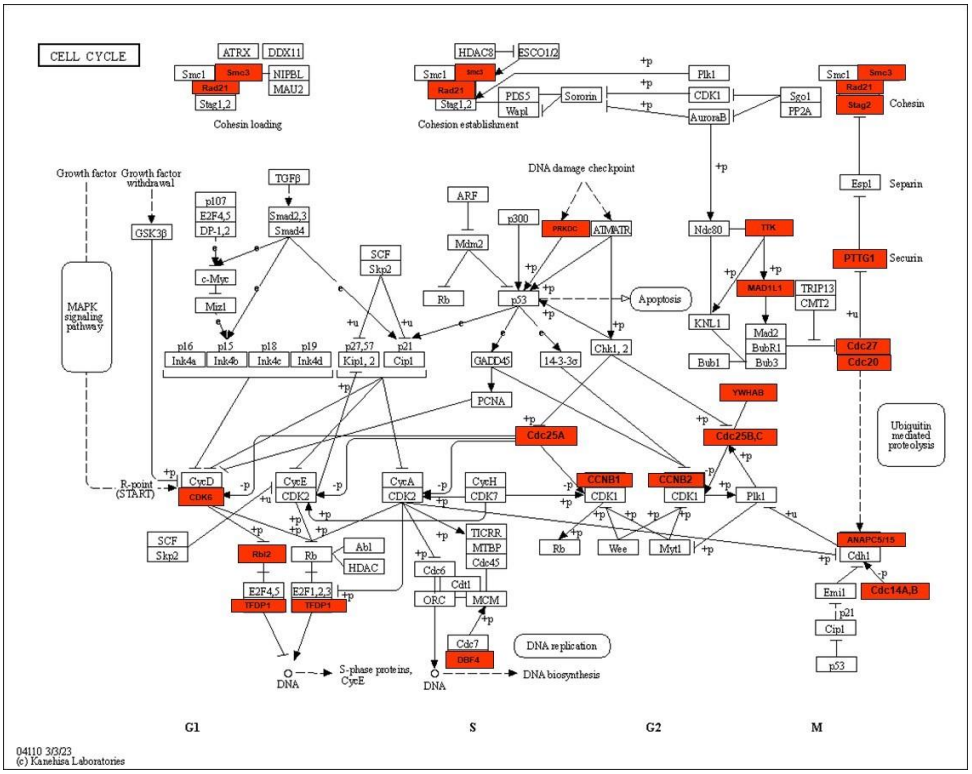

Figure 7. KEGG pathway analysis of differentially expressed genes on cell cycle pathway in AML patient group with high beta-catenin levels vs controls. Red color represents up-regulated in AML patients.

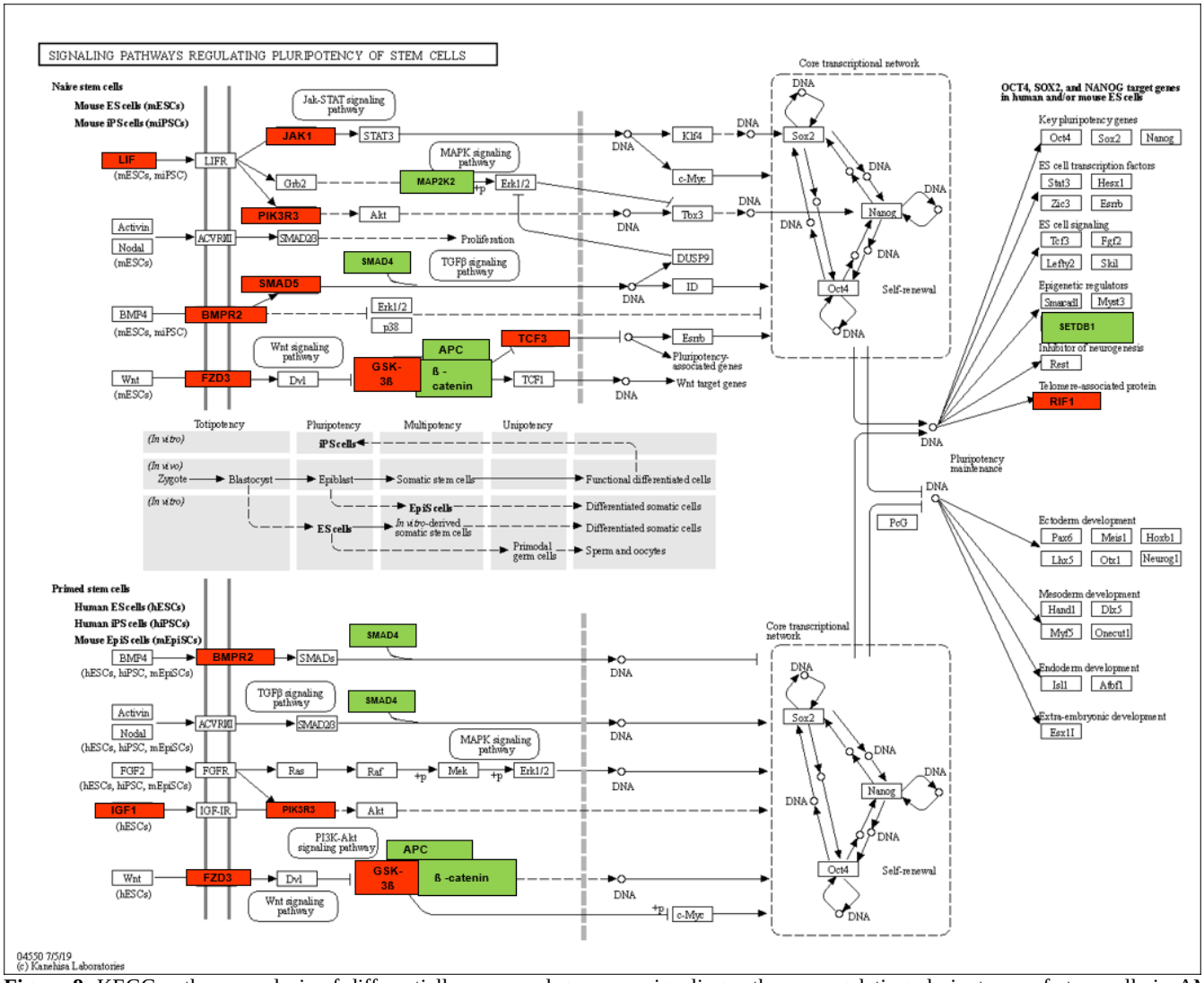

Figure 8. KEGG pathway analysis of differentially expressed genes on signaling pathways regulating pluripotency of stem cells in AML patient group with low beta-catenin levels vs controls. Red color represents up-regulated in AML, green color represents down-regulated in AML patients

Most of the DEGs were significantly enriched in cell cycle and regulating pluripotency of stem cells signaling pathways.

Cell Cycle Pathway. In our study, we observed differentially expression of many gene groups related with the cell cycle pathway in the AML group with high beta-catenin levels compared to controls (**Figure 7**). The expression of the genes *ANAPC15*, *YWHAB*, *PRKDC*, *TTK*, *CDC25C*, *SMC3*, *CDC25A*, *CDC14A*, *CDC25B*, *CDC14B*, *RBL2*, *CDC20*, *CCNB2*, *CCNB1*, *DBF4*, *STAG2*, *TFDP1*, *CDK6*, *PTTG1*, *RAD21*, *CDC27*, *ANAPC5*, *MAD1L1* in this pathway increased in the AML group with high beta-catenin levels

Signaling Pathways Regulating Pluripotency of Stem Cells. Another pathway in which genes are enriched in significant amounts is the regulating pluripotency of stem cells pathway (**Figure 8**). We compared the expression of genes with increased and decreased expression in this pathway between the AML patient group with low beta-catenin levels and controls. The 7 downregulated significant genes were *MAPK11*, *SMAD4*, *MAP2K2*, *APC*, *SETDB1*, *CTNNB1*, *PIK3R1* and 11 upregulated significant genes were *GSK3B*, *FZD3*, *BMPR2*, *RIF1*, *LIF*, *PIK3R3*, *TCF3*, *IGF1*, *IL6ST*, *SMAD5*, *JAK1*.

Protein-Protein İnteraction (PPI) Network Analysis. Further, we performed to better understand the functional modules in the PPI networks of the DEGs unique to AML patients in order to identify the beta-catenin -related key genes for this disease. The creation of a protein interaction network allows for the rapid analysis of gene interactions. The PPI network of DEGs identified in CD34+ cells of AML patients with high and low beta-catenin levels compared to healthy controls was analyzed using the STRING online database and Cytohubba plugin in Cytoscape software. Only in AML patients with high beta-catenin levels (high vs cont) were 722 nodes and 1970 edges, including 1194 up-regulated genes, and 614 nodes and 1463 edges, comprising 1120

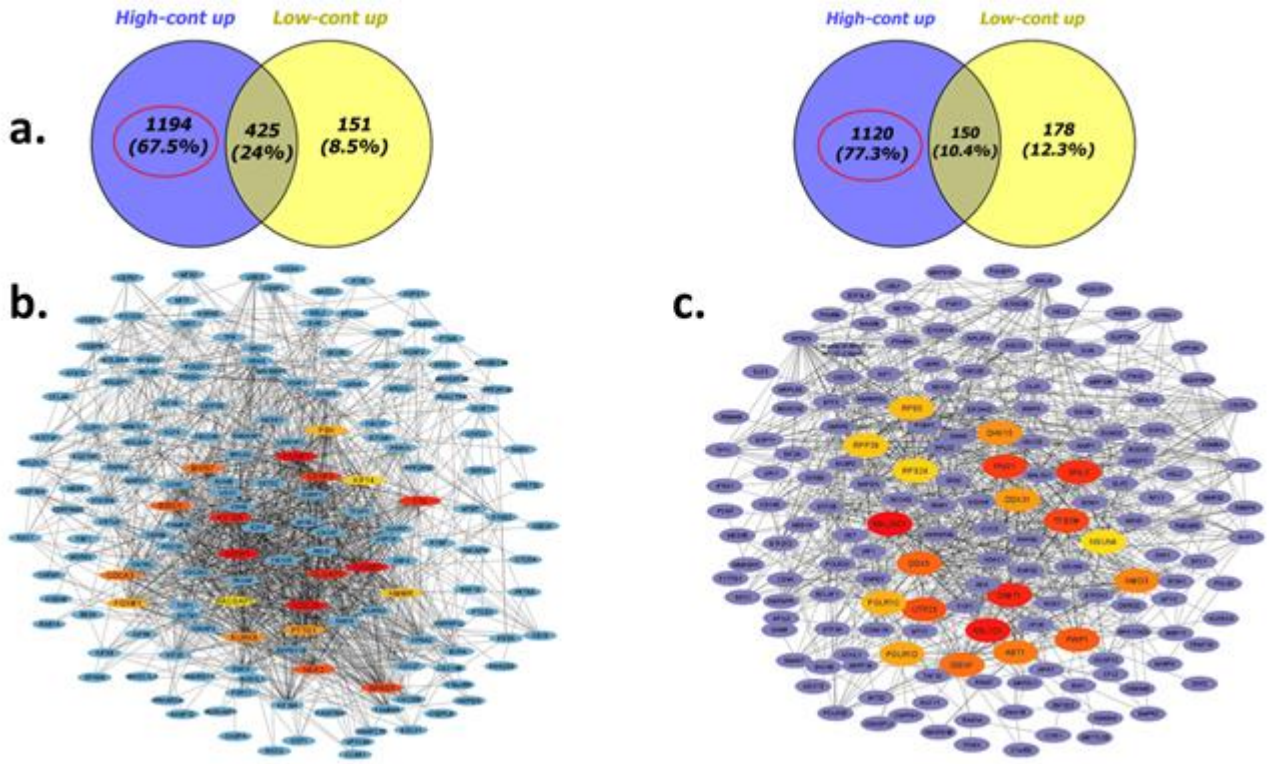

Figure 9. Venn diagram, PPI network of DEGs in High vs control **a.** Venn diagram of the DEGs, **b.** PPI network constructed with the 1194 up-regulated DEGs by the STRING, **c.** 1120 down-regulated DEGs were visualized by the STRING. Circles define genes, lines show protein interaction between genes. PPI, protein-protein interaction; DEGs, differentially expressed genes.

down-regulated genes, identified. 38 nodes and 32 edges, including 151 up-regulated genes, 46 nodes and 45 edges, including 178 down-regulated genes, were identified only in AML patients with low beta-catenin levels (low vs cont).

In the PPI network, hub genes were identified based on their degree and MCC (maximum correlation criterion) score. Following these analyses, it was discovered that 20 hub genes with different expression levels in both groups (high vs cont - low vs cont) played a critical role. Additionally, when networks are examined after PPI analysis, of the 20 hub (key) genes with highest expression on cell cycle pathway that were identified in AML progression in the present study were: *CDC20*, *KIF20A*, *CCNB2*, *CENPE*, *AURKA*, *CCNB1*, *PBK*, *TTK*, *DLGAP5*, *CENPF*, *MKI67*, *SPAG5*, *NEK2*, *BIRC5*, *PTTG1*, *HJURP*, *FOXM1*, *HMMR*, *KIF14*, *ANLN* (High vs cont) (**Figure 9a-b**). 18 hub genes involved in ribosome biogenesis, (*RSL24D1*, *PNO1*, *DIEXF*, *RSL1D1*, *PWP1*, *GNL3*, *NMD3*, *DHX15*, *UTP23*, *RPS5*, *RPS23*, *RPS24*, *DIMT1*, *RPL15*, *RPL37A*, *RPL22*, *C18orf32*, *DDX31*) and 2 hub genes (*TFB2M*, *BTF3*) acting as transcription factors were down-regulated in AML group with high beta-catenin level (High vs cont) (**Figure 9a-c**).

The highest node degree was found in four hub genes with high expression involved in DNA repair (*BLM*, *FANCD2*, *FANCI*, *EME1*) and four other genes involved in porphyrin metabolism (*PPOX*, *FECH*, *ABCB6*, *UROS)*. The up-regulated *TFRC*, *FTH1* hub genes involved in ferroptosis, as well as *BDNF*, *CDH2*, *GYPA* and *IL17* involved in hematopoietic cell lineage, were identified to have a central role in AML group with low beta-catenin level. *IGF1*, *PIK3R3*, and *GSK3ß* hub genes are all able to regulate stem cell pluripotency. *TLE1*, *BCLAF1*, and *RBPJ* genes interact as transcription factors. All these hub genes had high expression in AML group with low beta-catenin level (**Figure 10a-b**). Among the down-regulated hub genes, CTNNB1 (beta-catenin), *HIST1H2BD*, *KAT5*, *HIST1H2BK*, *PIK3R1*, *INPP5D*, *H3F3A*, *RUNX2*, *DOK1*, *RAB3GAP1*, *NCK2*, *DCTN4*, *NUDT3*, *KDM4B*, *EXOC7*, *VAMP4*, *OGT*, *ING4*, *ARRB1*, *MAP2K2* had the highest node degree in AML group with low beta-catenin level. These genes had central roles in multiple pathways, including stem cell pluripotency, epigenetic regulation, PI3K/AKT and MAPK signaling pathway, membrane trafficking pathway, GTPase activity, tumor supressor, metabolic signaling pathway (Low vs cont) (**Figure 10a-c**).

To identify hub genes in this PPI network, the important module was evaluated using the Cytohubba software plug-in MCODE. Analysis of up-regulated genes found 305 edges and 26 nodes in the high vs control group. There were 137 edges and 23 nodes identified for down-regulated genes. Six clusters with high MCODE score (score>5) were investigated and hub genes were listed in **Table 4** (score>5) (High vs cont).

The first cluster with the highest MCODE score consisted of upregulated genes (*DLGAP5*, *TTK*, *AURKA*, *ANLN*, *BIRC5*, *HJURP*, *TROAP*, *SPAG5*, *FOXM1*,

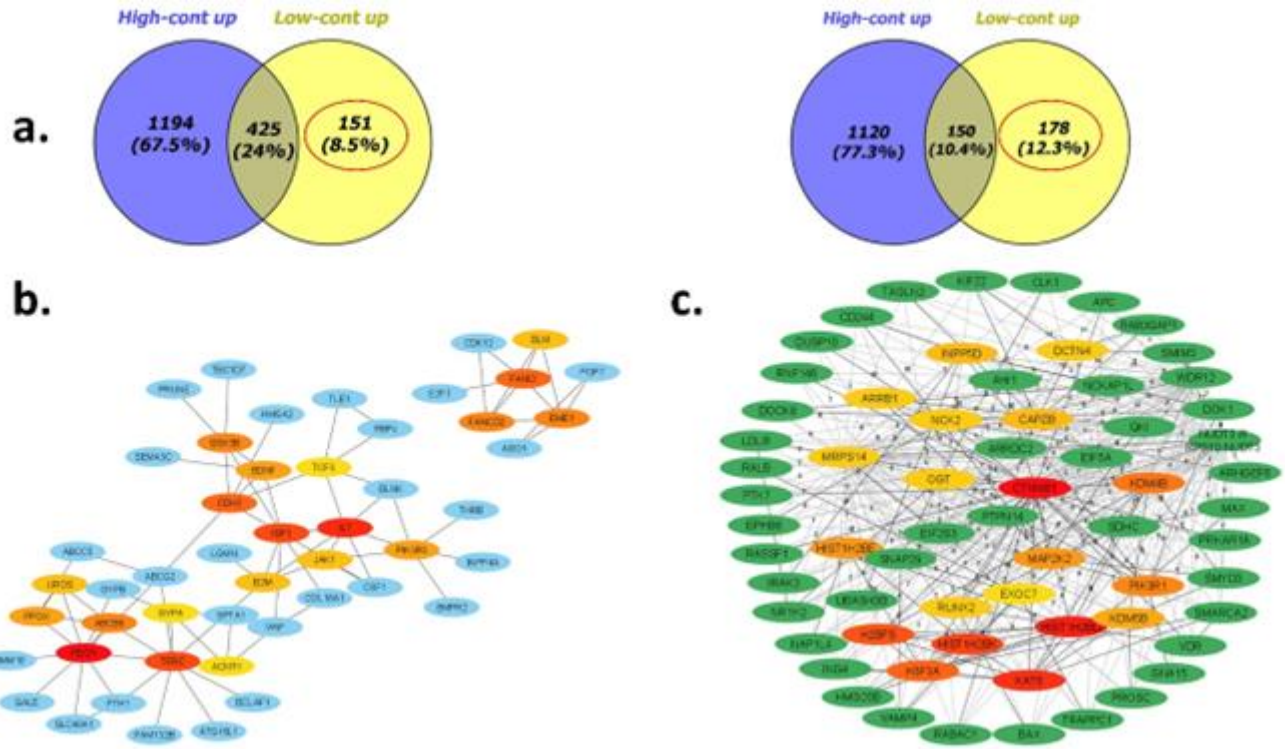

Figure 10. Venn diagram, PPI network of DEGs in Low vs control **a.** Venn diagram of the DEGs, **b.** PPI network constructed with the 151 up-regulated DEGs by the STRING, **b.** 178 down-regulated DEGs were visualized by the STRING. Circles define genes, lines show protein interaction between genes. PPI, protein-protein interaction; DEGs, differentially expressed genes.

Upregulated genes MCODE score		
Cluster	**Score**	**Node IDs**
1	**24,4**	***DLGAP5,TTK,AURKA,ANLN,BIRC5,HJURP,TROAP,SPAG5,FOXM1,RACGAP1, MKI67, KIF20A, CENPF, PTTG1,KIF14,CDC25C,CDC20,FAM64A,PBK, NEK2, CENPE, HMMR, CCNB1, KIF18A, CCNB2, CDCA***
2	7,75	***CWC27, SRRM2, RBM25, SF3B5, PRPF6, SF3B1, SRRM1, LSM5, RBM22***
3	7	***WDR36, KRR1, PAK1IP1, ESF1, GNL3L, DDX18, POLR1B***
4	7	***MRP63, MRPS16, MRPL28, MRPL35, AURKAIP1, MRPL30, MRPS2***
5	6,545	***TAF2,MGA,GTF2H3,RBBP5,TAF1,TAF3,GTF2F1,KANSL1,GTF2H2,SENP3,HCF C1, GTF2A2***
6	5,6	***CTSG, AZU1, DEFA4, ELANE, MPO, BPI***
Downregulated genes MCODE score		
Cluster	**Score**	**Node IDs**
1	**12,455**	***RPS23, TPT1, RPS5, BTF3, NMD3, PWP1, DDX31, RPL17, TFB2M, RSL1D1, PNO1, DIEXF, BTF3L4, RSL24D1, DHX15, RPL22, RPL37A, RRP36, GNL3, UTP23, RPL15, RPS24, DIMT1***
2	8	***STX12, VAMP3, NAPB, STX6, SEC22B, STX16, NAPG, STX4***
3	7	***RING1, ANAPC16, CDC26, PHC1, CDC16, PHC3, ANAPC10***
4	6,667	***MSH6, MSH2, XRCC4, ERCC1, XRCC5, C9orf142, MLH3***
5	6,571	***SNRPE, NAA38, MED11, SNW1, CWC25, SF3B3, CDK8, CDC5L, MED28, MED23, SDE2, MED14, SF3A1, MED30, CDC40***
6	5,882	***B2M, NCK1, WIPF1, IRF1, NFKB1, PPP3CA, ARPC5, MORF4L1, CYCS, RFWD2, PSME1, CNEP1R1, PSMB2, PSMA1, MTMR1, PPP2R2A, PSMB4, PPP2CB, PSMB9, PIKFYVE, ARPC3, ACAD8, ABI1, ZNFX1, SMAD2, PIK3C2B, HLA-G, INPP5F, HLA-B, CCNT2, POLR1D, POLR2K, SYNJ2, GTF3A, POLR3D, HLA-DRA, CCNY, PI4KA, INPP5B, HLA-C, SIN3A, CDK12, RBBP4, HDAC1, FAM60A, CDK13, STAT1, CCND2, ING1, PI4KB, BRMS1L, HLA-F***

Table 4. Significant module in the PPI having an MCODE score>5 in AML patient group with high beta-catenin levels vs controls.

RACGAP1, MKI67, KIF20A, CENPF, PTTG1, KIF14, CDC25C, CDC20, FAM64A, PBK, NEK2, CENPE, HMMR, CCNB1, KIF18A, CCNB2, CDCA) (**Supp. Figure 4**), which were primarily associated with the cell

cycle pathway. On the other hand, the downregulated genes (*RPS23, TPT1, RPS5, BTF3, NMD3, PWP1, DDX31, C18orf32 /// RPL17 /// RPL17-C18orf32, TFB2M, RSL1D1, PNO1, DIEXF, BTF3L4, RSL24D1, DHX15, RPL22, RPL37A, RRP36, GNL3, UTP23, RPL15, RPS24, DIMT1*) (**Supp. Figure 4**) were mainly associated with ribosome biogenesis and transcription factors (High vs cont).

In the low vs control group, two clusters were selected for genes with high scores (score>3) identified through MCODE analysis, representing upregulated genes, while a single cluster was chosen for downregulated genes, which is the most significant cluster (**Table 5**). The upregulated genes in cluster 1 were significantly enriched in DNA repair. Additionally, the genes in cluster 2 were predominantly associated with porphyrin metabolism (**Supp. Figure 5**). Furthermore, the downregulated genes in cluster 1 were primarily enriched in the regulation of actin filaments, DNA repair, and GTPase activity (**Supp. Figure 5**). The detailed information about the top 3 clusters was shown in **Table 5**.

Prognostic Analysis of the Hub Genes. Survival analysis was performed on 20 prominent genes that were enriched in both KEGG and GO pathways, as well as between hub genes, using the GEPIA online resource database (**Figure 11**).

Table 5. Significant module in the PPI having an MCODE score>3 in AML patient group with low beta-catenin levels vs controls.

Upregulated genes MCODE score		
Cluster	**Score**	**Node IDs**
1	4	***BLM, FANCI, EME1, FANCD2***
2	4	***PPOX, UROS, FECH, ABCB6***
Downregulated genes MCODE score		
Cluster	**Score**	**Node IDs**
1	3	***CAPZB, DCTN4, RAB3GAP1***

With the use of the GEPIA database, survival analysis was performed to analyze the relationship between the prognosis of AML patients and the expression of genes related to beta-catenin that were significant in our study. According to the findings, there may be a relationship between patient prognosis and expression differences because seven genes expression levels were found to be strongly correlated with the overall survival (OS) of AML patients. TTK, HJURP, KIF14, BTF3, C18orf32/RPL17, RSL1D1, and ELOV6 genes have been proposed as potential prognostic markers for AML patients with high beta-catenin levels (**Figure 11**).

Validation Microarray data. To validate the microarray analysis results, genes were selected from among the most differentially expressed genes in our dataset. Among the genes of interest, three genes (Grb-2, Klf4,

Table 6. 20 hub genes with high and low expression levels in High vs Control and Low vs Control groups.

High vs Control up	**High vs Control down**	**Down vs Control up**	**Down vs Control down**
CDC20	*RSL24D1*	*BLM*	*CTNNB1 (beta-catenin)*
KIF20A	*PNO1*	*FANCD2*	*HIST1H2BD*
CCNB2	*DIEXF*	*FANCI*	*KAT5*
CENPE	*RSL1D1*	*EME1*	*HIST1H2BK*
AURKA	*PWP1*	*PPOX*	*PIK3R1*
CCNB1	*GNL3*	*FECH*	*INPP5D*
PBK	*NMD3*	*ABCB6*	*H3F3A*
TTK	*DHX15*	*UROS*	*RUNX2*
DLGAP5	*UTP23*	*TFRC*	*DOK1*
CENPF	*RPS5*	*FTH1*	*RAB3GAP1*
MKI67	*RPS23*	*BDNF*	*NCK2*
SPAG5	*RPS24*	*CDH2*	*DCTN4*
NEK2	*DIMT1*	*GYPA*	*NUDT3*
BIRC5	*RPL15*	*IL17*	*KDM4B*
PTTG1	*RPL37A*	*IGF1*	*EXOC7*
HJURP	*RPL22*	*PIK3R3*	*VAMP4*
FOXM1	*C18orf32*	*GSK3ß*	*OGT*
HMMR	*DDX31*	*TLE1*	*ING4*
KIF14	*TFB2M*	*BCLAF1*	*ARRB1*
ANLN	*BTF3*	*RBPJ*	*MAP2K2*

Gsk3ß) showed parallel expression patterns with the microarray data and exhibited statistical significance (**Supp. Figure 3**).

Discussion. In spite of the development of treatment possibilities, AML is still the most common haematological malignancy in terms of incidence and cancer-related deaths.[21] It has also been known for over 15 years that beta-catenin dysregulation[15] in AML promotes the development, protection and drug resistance of leukaemia stem cells. Despite this knowledge, β-catenin inhibitors have not yet reached the clinic.[7]

Using genomic tools, significant progress has been made in studying AML at the molecular level. Most AML gene expression profile (GEP) studies, however,

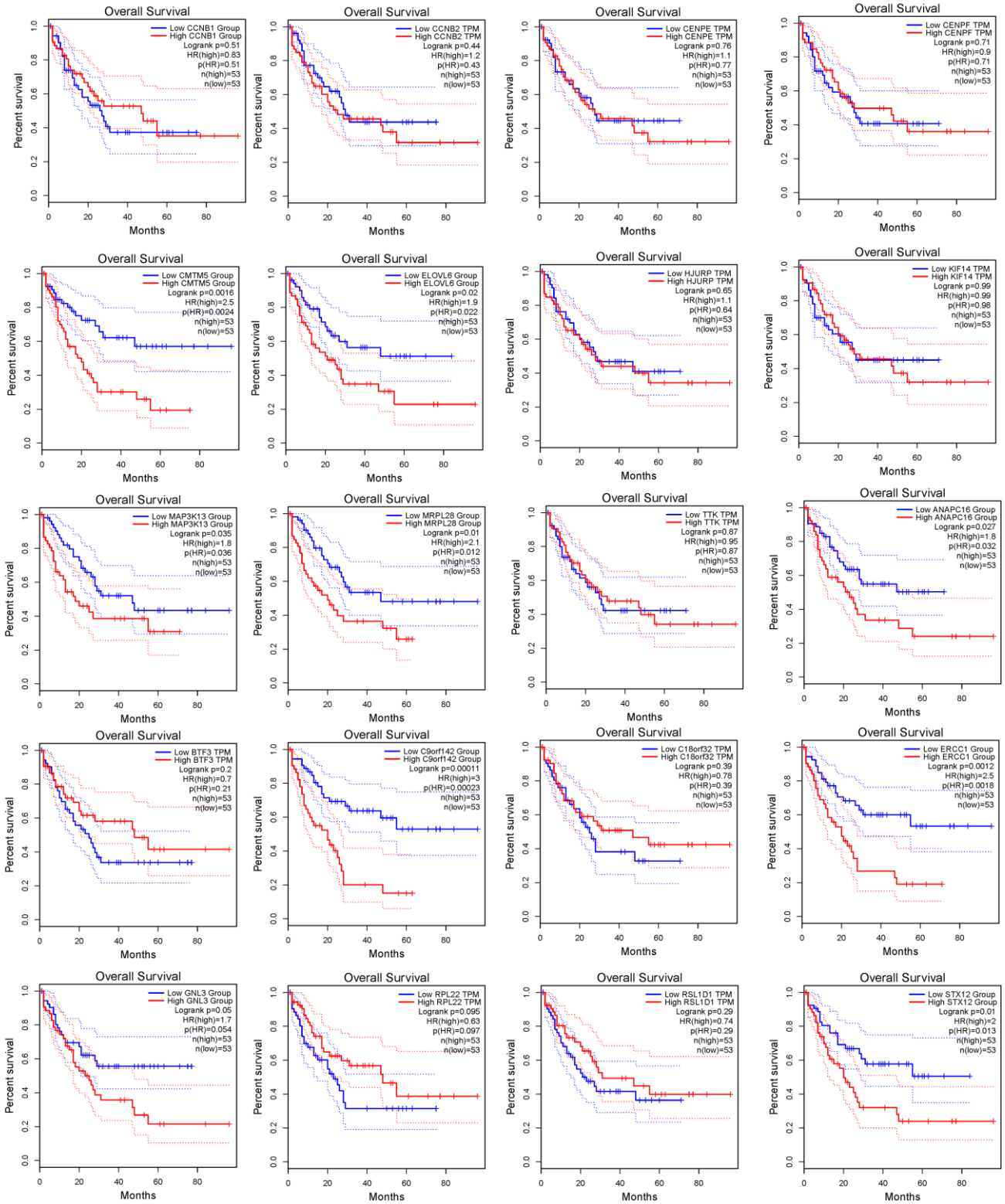

Figure 11. Relationship between target gene expression and survival of patients with AML with high beta-catenin level (drawn by GEPIA).

have been performed with the total AML mononuclear cell (MNC) fraction.[22] Although many studies have been published in this area, our research is the first transcriptomic analysis to investigate beta-catenin-related genes in CD34+ cells of AML population.

Our study has many novel features. First, we determined beta-catenin gene expression levels in CD34+ cells isolated from AML patients compared to the control group. Second, we found that beta-catenin levels varied between AML patients. Third, we evaluated transcriptional changes between AML samples (AML patient group with high and low beta catenin levels). We showed for the first time that the transcriptome profiles of AML samples show different molecular signature profiles according to beta-catenin levels. Finally, we identified genes that were differentially expressed among AML patients grouped according to beta-catenin levels, and when these genes were combined with genes in the control group, we identified for the first time genes and pathways related to beta-catenin in AML.

In our study, to identify beta-catenin-associated genes in the AML group, we focused on 2314 genes, excluding DEGs commonly detected in the venn diagram of the two different groups: high vs control and low vs control. Thus, in this study, we aimed to understand the molecular effects of the AML process by especially analysing patients with high beta-catenin levels (high vs cont) and to identify possible beta-catenin-related biomarkers that may be involved in disease progression.

We also performed protein-protein interaction networks using 2314 genes and selected 20 hub genes that are involved in beta-catenin related AML. These hub genes included "*CDC20, KIF20A, CCNB2, CENPE, AURKA, CCNB1, PBK, TTK, DLGAP5, CENPF, MKI67, SPAG5, NEK2, BIRC5, PTTG1, HJURP, FOXM1, HMMR, KIF14, ANLN*" which significantly enriched in cell cycle pathway. It showed that these hub genes were significantly upregulated in patients with high beta-catenin levels compared to controls. It was shown that the hub genes "*RSL24D1, PNO1, DIEXF, RSL1D1, PWP1, GNL3, NMD3, DHX15, UTP23, RPS5, RPS23, RPS24, DIMT1, RPL15, RPL37A, RPL22, RPL17-C18orf32, DDX31, TFB2M, BTF3*" which showed decreased expression, played a role in pathways such as ribosome biogenesis and transcription factors in AML patients with high beta-catenin levels.

The link between cancer development and dysregulation of cellular proliferation is well documented and this is confirmed by the growing list of genetic or transcriptional changes affecting the main components of the cell cycle regulatory mechanisms in malignant cells. In addition, cell cycle proteins have emerged as a new category of potential therapeutic candidates. Many studies have investigated the mechanisms that control the correct segregation of sister chromatids during the final step of cell division. Cell division cycle 20 homologue (*CDC20*) is an important protein that regulates the cell cycle by controlling the correct separation of chromosomes during mitosis. The overexpression and/or oncogenic role of *CDC20* has been reported in several human solid tumours.[23] Approximately 20% of AML patients have chromosome segregation defects due to abnormal *CDC20* expression.[24] In our study, *CDC20* gene expression levels were found to be higher in AML patients compared to the control group, which is consistent with the studies in the literature. In addition, knock down of *CDC20* in colon cancer cells decreased the expression levels of beta-catenin target genes, leading to increased phosphorylation of beta-catenin. Thus, *CDC20* is responsible for regulating Wnt/beta-catenin signalling.[25] In accordance with these data reported by Hadjihannas et al., we also found high levels of *CDC20* expression in AML patients with high levels of beta catenin in our study. Another cell cycle protein, microtubule-associated motor protein Kinesin-like family member 20A (*KIF20A*) is involved in intracellular vesicular trafficking and is necessary for cytokinesis in cell division. *KIF20A* plays a role for developing of a number of malignancies. Morita et al., indicated that the aggressive proliferation of leukemic cells is related to the main role of *KIF20A* based on their findings with HL60 leukemic cells.[26] In parallel to the results of Morita et al., we also found that *KIF20A* expression was higher in AML patients with high levels of beta catenin as compared to controls.

The progression of the cell cycle is mainly controlled by cyclins and cyclin-dependent protein kinases (CDKs). Cyclin B1 (*CCNB1*), one of the key genes in our study, is an important cell cycle regulator protein that promotes mitosis and its aberrant expression in various human malignancies has been associated with poor prognosis. Abnormal *CCNB1* expression has been reported in the literature in the majority of human AML patients.[27] Cyclin B2 (CCNB2), a member of the cyclin family, which is also upregulated in human cancers (bladder, lung and breast),[28] was found in our study to be one of the hub genes in AML and is mainly involved in the G2/M transition.[29] Consistent with the literature, we identified increased *CCNB1* and *CCNB2* genes expression in AML patients with high beta-catenin levels compared to controls. Moreover, chemotherapy resistance and the progression of disease are significantly influenced by genes linked to mitosis. It is yet unclear how these genes show expression and what roles they play in drug-resistant AML patients. The researchers investigating into the roles of the spindle assembly checkpoint (SAC) genes; *CENPE*, *CENPF*, and *DLGAP5* in chemoresistant AML patients reported that these genes were linked to a poor prognosis and were increased in drug-resistant AML patients compared to drug-sensitive patients.[30] In parallel with the study by Shi

et al., we found that the expression levels of SAC genes were higher in AML patients with high beta-catenin levels. In addition, in a study with *DLGAP5*, one of the SAC genes, Chen et al., showed that in epithelial cancer (EC) cells, *DLGAP5* knockdown inactivates the Wnt/beta-catenin signaling pathway. Furthermore, it has been demonstrated that *DLGAP5* knockdown blocks the EC cells ability to proliferate by disrupting the Wnt/beta-catenin signaling pathway.[31]

As a member of the serine/threonine kinase family, Aurora kinase A (*AURKA*) controls mitosis, which is necessary for cell division. *AURKA* expression is significantly higher in various malignancies, including hematological malignancies.[32,33] *GSK-3β* is an Aurora kinase substrate.[34] In our study, *AURKA* expression was analysed to be higher in AML patients with high beta-catenin levels compared to the control group in accordance with the literature.

The expression of the novel mitotic kinase called PDZ-binding kinase (*PBK*) is dramatically downregulated during the terminal differentiation of HL-60 leukaemia cells and elevated in hematological malignancies.[35,36] Moreover, the role of beta-catenin in promoting the aggressive result of *PBK* expression in vitro is significant.[37] In our study, *PB*K expression levels were found to be elevated in AML patients with high beta-catenin levels in agreement with the literature. Monopolar spindle 1 (Mps1) kinase or TTK Protein Kinase (*TTK*), is the another mitotic kinase that is necessary for centrosome duplication and chromosomal orientation at the centromere during mitosis. Furthermore, an increasing number of study indicates that *TTK* is linked to a poor prognosis and malignant progression in several different cancers.[38] Furthermore, it was discovered that the mutant *CTNNB1* encoding beta-catenin is a distinct predictive therapeutic response biomarker for TTK inhibitors.[39] In our study, we found that especially in AML patients with high beta catenin levels, the levels of expression of the TTK gene that we associate with beta-catenin, were increased for the first time.

The cell cycle and marker of proliferation Ki-67 (*MKI67*) antigen expression are strongly associated. It is nearly always expressed throughout the mitosis in the cell cycle. When haematological malignancies are detected, the presence of the *MKI67* antigen may indicate active cell proliferation. Numerous studies investigated the expression of MKI67 in acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL), as well as the correlation between *MKI6*7 expression and prognosis and treatment outcome. *MKI67* in AML has been the subject of few investigations, and the findings of those that have been completed have not always been accurate.[40,41] In our study, we found that *MKI67* gene expression levels were higher in AML patients compared to the control group.

A protein found on the mitotic spindle is called sperm associated antigen 5 (SPAG5). According to recent studies, it acts as an oncogene and is overexpressed in a large number of human malignancies. Moreover, other upstream regulators, such as *GSK3β*, control *SPAG5*.[42] According to Gu et al., patients with newly diagnosed AML had significantly higher levels of *SPAG5* expression than both normal controls and patients with AML who observed complete remission.[43] Expression of *SPAG5*, a beta-catenin-related gene, was found to be high in the AML patient group with high beta-catenin levels in our study, in parallel with previous studies. Several human malignancies exhibit aberrant overexpression of two other proteins, NIMA-related kinase 2 (*NEK2*) and Human Baculoviral inhibitor of apoptosis repeat-con-taining 5 (*BIRC*5), which are important in the progression of the cell cycle and mitosis. Specifically, *NEK2* has been found to be a gene strongly linked to drug resistance, a rapid relapse and a poor prognosis in patients with various malignancies.[44-46] For the first time, Bai et al., demonstrated that in lung cancer cells, *NEK2* knockdown can deactivate the canonical Wnt/beta-catenin signaling pathway.[47] Furthermore, the study performed by Zhou et al., shown that *NEK2* enhances the potential of renal-cell carcinoma cells to proliferate, migrate, and invade by triggering the Wnt/beta-catenin signaling pathway.[48] Studies has indicated that in 60% of adult AML patient samples, *BIRC5* expression is detected. Complex cytogenetic aberrations in adult AML patients were associated with high expression of *BIRC5*.[49] Additionally, it has been demonstrated that *BIRC5* interacts with *AURKA* to inhibit *GSK3β,* which activates classical Wnt signaling.[50] In the course of our research, we discovered that AML patients (High vs. cont.) had higher expression levels of all 3 genes (*NEK2*, *BIRC5*, *AURKA*), that control the cell cycle and related to Wnt signaling, compared to controls. It is also known that Pituitary tumor-transforming gene 1 (*PTTG1*), an oncogene also referred to as securin, regulates the G1/S and G2/M phase transitions affecting the cell cycle. Most normal tissues have minimal or undetectable levels of *PTTG1* protein, however it is widely expressed in a variety of invasive tumors and hematopoietic malignancies.[51] Additionally, *PTTG1* plays a crucial role in controlling the Wnt pathway and the development of hepatic tumors by binding directly to beta-catenin and triggering the transcription of Wnt target genes.[52] The results of our study were consistent with previous studies, and *PTTG1* levels increased, especially in AML patients with higher beta-catenin levels in comparison to the control group.

Centromere protein A (*CENP-A*), which is necessary for chromosomal segregation during mitosis and cell cycle regulation, has Holliday junction recognition protein (*HJURP*) as a critical molecular chaperone. Abnormal up-regulation of *HJURP* expression

mediating Wnt/beta-catenin signaling is linked to poor clinical progression and prognosis. This has been observed in a number of human malignancies, including bladder, breast, hepatocellular (HCC), and non-small cell lung cancer (NSCLC).[53] For the first time, we suggest that AML patients had higher expression of the beta-catenin related *HJURP* gene, as our study indicated.

The proliferation-associated transcription factor, Forkhead box protein M1 (*F*OXM1), which was named the 2010 Molecule of the Year, enhances cell proliferation. *FOXM1* promotes entry into S-phase and M-phase, which accelerates the progression of the cell cycle.[54,55] Overexpression of *FOXM1* has been linked to the survival of many cancer types (lung, colon, prostate, liver, glioblastoma and blood cancers including ALL, myeloma) and is a sign of a poor prognosis for cancer patients.[56] It has also been shown that *FOXM1* expression levels in mesenchymal cells from patients affected by myeloid neoplasms play a role in promoting haematopoiesis.[57] According to recent studies, *FOXM1* suppression reduced the proliferation of AML leukemia cell lines. Furthermore, chemoresistance in AML has been discovered to be promoted by *FOXM1*, however its exact molecular mechanism is still unknown . Present studies indicate that overexpression of *FOXM1* increases the Wnt/beta-catenin signaling pathway by directly binding to beta-catenin, which keeps leukemic stem cells (LSCs) quiescent and promotes LSC self-renewal in AML.[58] Our findings were consistent with the study by Sheng et al., and analysed that *FOXM1* expression was highly expressed in our AML patients. Currently, studies has demonstrated that the cell cycle and division are affected by the Hyaluronan-Mediated Motility Receptor (*HMMR*), also called CD168. Moreover, *HMMR* protein has been linked to a variety of tumor types, including sarcoma, AML, lung and breast cancers. These findings demonstrated the critical role *HMMR* plays in cancer metastasis as well as the initiation and spread of malignancy.[59] We found that *HMMR* was significantly upregulated in AML patients with high beta-catenin levels, confirming the results of a previous studies.

Kinesin superfamily (KIFs) proteins are essential for molecular motors that facilitate the movement of organelles, vesicles, and chromosomes throughout the events of meiosis and mitosis in cells. Kinesin family member 14 (*KIF14*), a member of this family of microtubule-based motor proteins that plays important roles during cell cycle progression under basic conditions.[60] A worse prognosis has been estimated in cases of human gastric, epithelial ovarian, cervical, breast, and hepatocellular carcinomas where *KIF14* expression is elevated.[61] Our study is the first to identify a high level of expression of the beta-catenin-related *KIF14* gene in patients with AML. This is consistent with studies in various cancers in the literature. Another of our hub genes, Anillin *(ANLN)*, which is essential for cytokinesis, is elevated in the early stages of 17 cancers, and in more than ten tumors, higher expression of *ANLN* is associated with a poorer prognosis.[62] In our study, we found for the first time that AML patients with high levels of beta-catenin had higher expression of the *ANLN* gene.

Ribosome formation is stimulated by compounds that promote cell proliferation, even though the rate of ribosome biogenesis controls the progression of the cell cycle.[63] Moreover, a number of genes directly or indirectly involved in ribosome biosynthesis have their expression controlled by Wnt signaling.[64] For the first time, genes involved in ribosome biogenesis whose expression levels decreased in the patient compared to the control in our study were *RSL1D1, PNO1, DIEXF*, and *RSL24D1*, which are thought to be beta-catenin related genes in AML but have not been studied in the literature in the context of AML as potential biomarkers. Ribosomal L1 domain-containing protein 1 (*RSL1D1*), one of the hub genes in ribosome biogenesis in our study, is a nucleolar protein that is essential in cell proliferation.[65] As shown in the study by Liu et al., *RSL1D1* expression was found to be significantly increased especially in tumours from patients with colorectal cancer and high *RSL1D1* expression was associated with a poorer survival in these patients.[66] On the other hand, not much research has been done on RNA-binding protein partner of NOB1 (*PNO1)*'s role in malignancies related to ribosomes. Numerous investigations have demonstrated the significance of *PNO1* in the formation and carcinogenesis of tumor, as our study demonstrated.[67-69]

Blood-based cancer detection may be made possible by tumour-educated platelets (TEPs). Ge et al.'s study aimed to find diagnostic TEP genes involved in carcinogenesis; among these TEP genes, ribosomal L24 domain containing1 (*RSL24D1*), one of our study's hub genes, was identified in AML patients with high beta-catenin levels.[70] *RSL24D1* alterations has been linked to developed non-small cell lung cancer, according to a recent genome-wide methylation profile research. The results of this study showed that, as compared to healthy individuals, *RSL24D1* was adversely related with early-stage malignancies, such as colorectal, pancreatic, hepatobiliary, lung, and breast cancers.[71]

It is possible that new strategies for inhibiting pathways are still being discovered because many tumors continue to carry wild-type p53 following being treated with p53 inhibitors. A new p53 inhibitor in zebrafish was recently found to be digestive organ expansion factor (*DIEXF*).[72]

Periodic tryptophan protein 1, also known as endonuclein (*PWP1*), a nucleolar protein *GNL3*, and the nuclear export adaptor protein *NMD3* were found to be among the beta-catenin related genes in AML for the first time in our study, and their expression levels were

decreased in AML patients compared to controls. Several studies show that aberrant expression or activity of the RNA-binding protein (RBP) is a characteristic of aggressive types of leukemia and a driver of its progression. Although there is growing evidence that DEAH-box RNA helicase *(DHX15)* plays a significant role in human malignancies, it is still not entirely understood how tumorigenesis is altered by aberrant *DHX15* expression and whether it results in dysregulated RNA splicing.[73] It has been demonstrated that when AML patients have remission, *DHX15* is downregulated. According to these findings, *DHX15* deficiency causes DNA damage that results in cell cycle arrest and death, which further impairs the ability of leukemia cell lines to proliferate.[74] In our study, *DHX15* expression decreased in AML patients with high beta-catenin levels compared to the control group, in accordance with the study by Pan et al.

Many ribosomal proteins (RPs) have also been shown to be differentially expressed in cancer. Both the early and late stages of colorectal cancer were shown to overexpress *RPS23*. Additionally, human malignant prostate tissues were discovered to have an increased expression of *RPS24*. However, in some cases of colon cancer, RPs are down-regulated. The progression of tumors has been linked to the downregulation of *RPS5* in colon cancer. According to Yan et al., pancreatic cancer patients with low expression of *RPL15* had a worse prognosis and were at greater risk to show cell invasion and metastasis. We found that the expression levels of *RPS23*, *RPS24*, *RPS5*, and *RPL15* genes, which we suggested to be related to beta-catenin and might be biomarkers in AML, were lower in the AML patient group than in the control group. NSCLC has been shown to have down-regulated *RPL22*, indicating that low expression of *RPL22* could be linked to the carcinogenesis of NSCLC. Human myelodysplastic syndrome (MDS) and AML have been reported to frequently indicate decreased ribosomal protein *RPL22* expression; this reduction is linked to worse outcomes.[75] Our research and the results of Khoury et al.'s study are consistent, and we have also found decreased *RPL22* gene expression in AML patients. While Urwanisch et al., shown that AML patients had overexpressed *RPL37A* at the transcriptional level, our study indicated that AML patients showed decreased *RPL37A* expression when compared to the control group.[76]

A nucleolar protein called UTP23 Small Subunit Processome Component (*UTP23)* is necessary for the production of ribosomes. Studies has shown that samples of breast cancer patients express *UTP23* at significantly higher levels than samples of healthy individuals, and that higher *UTP23* levels are strongly correlated with a poor prognosis.[77] However, Fu et al. found that patients with ovarian cancer who had down-regulated *UTP23* had a worse prognosis.[78] *UTP23* gene expression decreased in our AML patients compared to the control group, which is parallel with the findings of Fu et al., and in contrast to Li et al. The rRNA-modifying enzyme, dimethyladenosine transferase 1 (*DIMT1*) is important in the production of ribosomes; ablation of this gene results in the disruption of ribosome biogenesis and is fatal to human cells. According to reported experimental results, AML cell lines were more sensitive to *DIMT1* deletion than the solid tumor cell lines that were examined. After *DIMT1* eliminated, ribosome profiling of AML cells showed dysregulated transcript subsets, indicating that *DIMT1* may influence a variety of cancer pathways and malignancies, including leukemia.[79] Our findings indicated that *DIMT3* gene expression was lower in AML patients as compared to the control group, which is similar to results in the literature. *RPL17-C18orf32*, another ribosomal protein, was decreased in AML patients with high beta-catenin levels, compared to the control group in our study for the first time.

Recent studies have reported high expression of various members of the DEAD box proteins (DDX) family in some cancer types.[80] The overexpression of *DDX31*, a member of the DDX family linked in cellular processes involving RNA secondary structure alterations, has been shown in both in vitro and in vivo studies to improve the migration, proliferation, and viability of pancreatic cancer cells.[81] Furthermore, it has been demonstrated that *DDX31* is absent in 25% of pancreatic cancer patients.[82] Unlike Liu et al.'s study, a decrease in *DDX31* gene expression was observed in our AML patients.

The expression of mitochondrial genes is influenced by genes involved in the mitochondrial transcription machinery (MTM). *TFB2M*, one of the MTM genes, is increased in AML patients compared to healthy donors, according to a single study that has been reported in the literature. Normal cytogenetic status, NPM1 mutations, and an elevated peripheral blood blast percentage have all been linked to upregulation of one or more MTM genes.[83] However, in contrast to this study, in our study, *TFB2M* is downregulated in AML patients with high beta-catenin levels.

Basic transcription factor 3 (*BTF3*) a part of the complete transcription machinery, forms a stable complex with RNA polymerases to function as a transcriptional initiation factor from proximal promoter sites. There is growing evidence that *BTF3* regulates apoptosis and is aberrantly expressed in a variety of human malignancies, including colorectal cancers.[84] The *BTF3* gene, which we identified to be associated with beta-catenin, showed a decrease in expression in our study's AML patients for the first time.

Conclusions. In conclusion, our study identified DEGs that are significantly associated with poor overall survival of AML patients, both in the KEGG and GO

pathways and among hub genes. In this case, *TTK*, *HJURP*, *KIF14*, *ELOVL6*, *RPL17-C18orf32*, *RSL1D1* and *BTF3* genes are proposed for the first time as a target gene thought to be associated with beta-catenin in AML, and since expression differences in these genes reduce survival rates, we can propose these genes as a prognostic marker associated with poor prognosis. In addition, the fatty acid elongase *ELOVL6*, which is very important for haematopoietic stem cells, was the most up-regulated gene in AML samples with high beta-catenin levels in our study and was associated with poor prognosis in human AML patients for the first time.

Moreover, the progression of AML disease and its response to therapy may be significantly influenced by the cell cycle, ribosome biogenesis, transcription factors, and lipid metabolism pathways. For the purpose of treating AML specifically, these pathways and genes may therefore be potential therapeutic targets.

In brief, our results have identified potential prognostic markers for AML and shed light on the mechanism of AML. These genes may serve as potential targets for leukaemia, although further clinical validation is needed in addition to our analyses.

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