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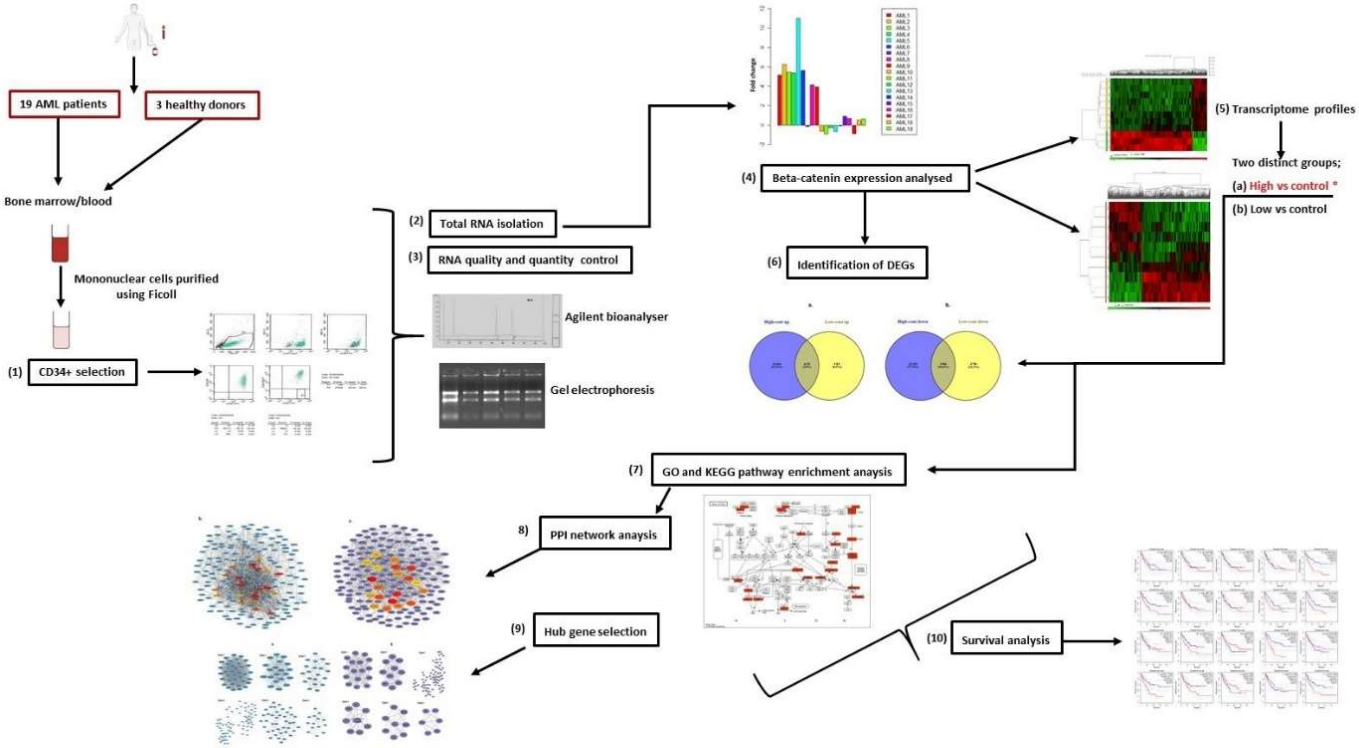
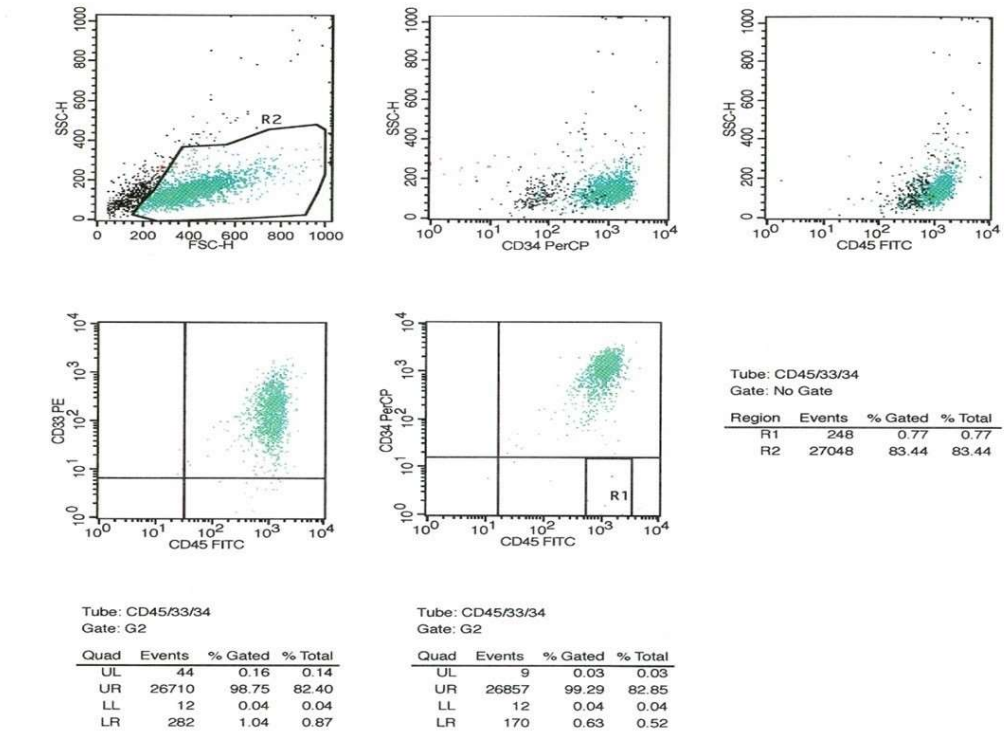
Original Article

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

Supplementary materials.

Supplementary Figure 1. Schematic diagram of this study.



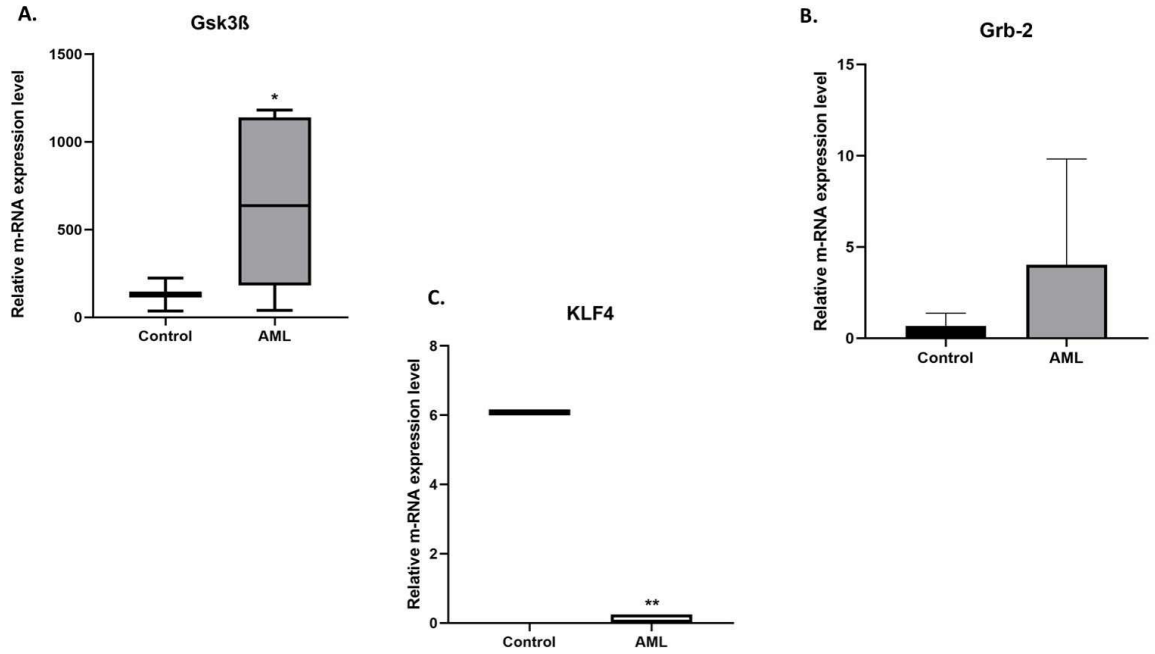


Supplementary Figure 2. CD34+ hematopoietic cells isolated from bone marrow/blood of AML patients (n=19) were cultured for 24 hours. At the end of the 24th hour, the cells were collected and analysed by flow cytometry using CD33, CD34, CD45 antibodies, which are surface markers of differentiation. The results of the analysis showed that 98% of the cells cultured for 24 hours were blastic cells, i.e. they retained the CD34+ phenotype and did not undergo differentiation. It was found that CD34 and CD33 expression was present in 98% of the cells, supporting the myelomonocytic origin of the cells. CD45 expression was weaker due to the very small number of possible lymphoid cells.

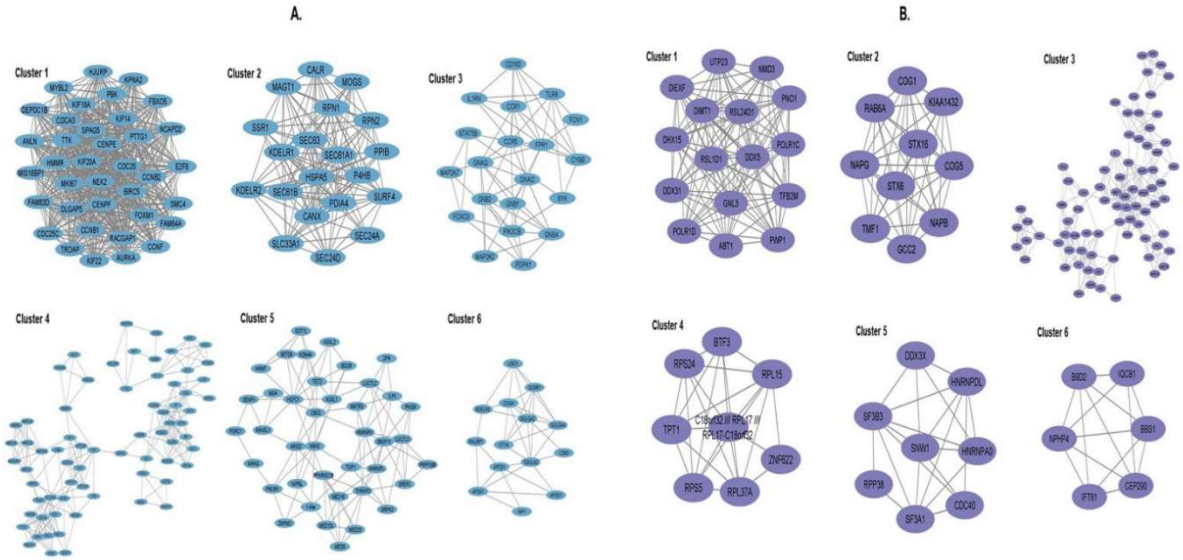
Supplementary Table 1. Patient characteristics according to beta-catenin expression levels.

	Beta-catenin (+) (n=8)	**Beta-catenin (-) (n=7)**	**Beta-catenin no change (n=4)**	**Total (n=19)**	**P value**
Age					
≤60	5 (62.5)	6 (85.7)	1 (25)	12 (63.2)	
≥60	3 (37.5)	1 (14.3)	3 (75)	7 (36.8)	NS
Mean	53	46	68	54	
Gender					
Female	3 (37.5)	3 (42.9)	1 (25)	7 (36.8)	
Male	5 (62.5)	4 (57.1)	3 (75)	12 (63.2)	
Leukocytes (median, range)	37 000 (2400-71 600)	74 957 (36 500-113 000)	26 100 (1000-51 200)	48 689	NS
FAB subtype					
M0	1 (14.3)	0 (0)	1 (33.3)	2 (10.5)	
M1	0 (0)	1 (20)	0 (0)	1 (5.2)	
M2	1 (14.3)	1 (20)	1 (33.3)	3 (15.7)	
M3	0 (0)	1 (20)	0 (0)	1 (5.2)	NS
M4	4 (57.1)	2 (28.5)	0 (0)	6 (31.5)	
M5	0 (0)	1 (20)	0 (0)	1 (5.2)	
NA (%)	2 (25)	1 (20)	2 (50)	5 (26.3)	
CD34% (Median, range)	73.83 (49-95)	43.26 (15-71)	43.33 (3-86)	55.50	NS
Survival rate%	4 (50)	5 (71.4)	1 (25)	10 (52.6)	
Dead	4 (50)	2 (28.5)	3 (75)	9 (47.3)	NS
Karyotype%					
Favorable	0 (0)	2 (28.5)	0 (0)	2 (10.5)	
Intermediate	3 (37.5)	1 (14.2)	1 (25)	5 (26.3)	
Adverse	2 (25)	3 (42.8)	2 (50)	7 (36.8)	NS
NA (%)	3 (37.5)	1 (14.2)	1 (25)	5 (26.3)	

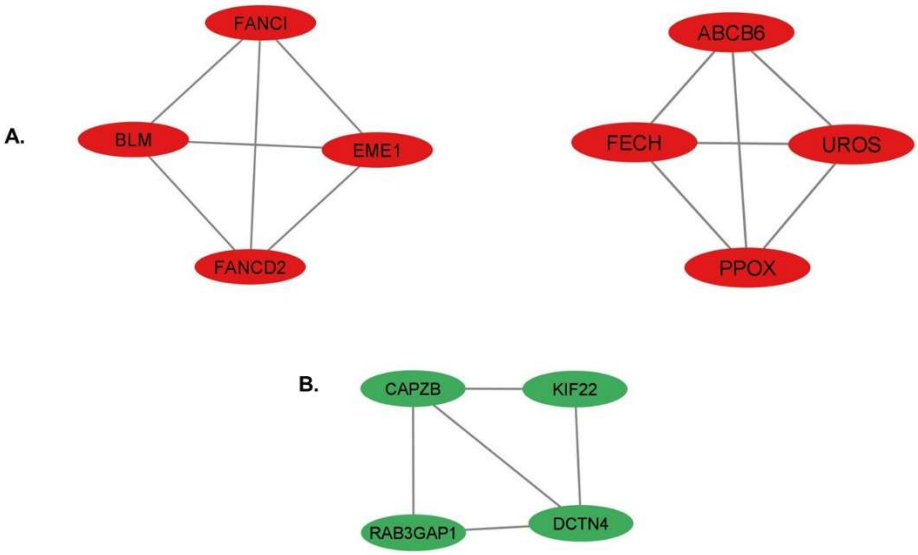
*NS: p>0.05. **NA: missing information. ***Karyotypes were categorised as favourable (t(15;17) and inv(16)), intermediate (normal karyotype and monosomal karyotype, del(9), del(16)) and adverse (all others, including complex karyotypes). Mann-Whitney (medians) was used to compare variables and p values are shown. Induction chemotherapy ("3+7") was used in the majority of AML patients in our study. The drugs used in the standard induction therapy are a combination of cytosine arabinoside (Ara-C) and an anthracycline. The anthracyclines used were idarubicin, mitoxantrone or daunorubicin. Hydroxyurea has also been used in older patients.



Supplementary Figure 3. Comparison of QPCR gene expression levels between control vs. AML groups. **A.** Gsk3ß gene expression levels in AML patient group with low beta-catenin levels compared to controls (p=0.0113*) (Low vs control); **B.** Grb-2 gene expression levels in AML patient group with high beta-catenin levels compared to controls (p=0.67) (High vs control); **C.** KLF4 gene expression levels in AML patient group with high beta-catenin levels compared to controls (p<0,0001) (High vs control). Statistical software Graph Pad Prism used for the assessment. The one sample t-test was used to compare the gene expression values between patient and control groups.



Supplementary Figure 4. Top 6 modules from the PPI interaction networks A. Upregulated genes, B. Downregulated genes was analyzed by MCODE plugin in cytoscape software in High vs control. It was observed that the genes with higher expression (first 20 hub genes) and the genes with the lower expression (first 20 hub genes) are grouped into 6 clusters (MCODE score>5). It was observed that 20 of the 26 genes upregulated in the first cluster with the highest MCODE score among these clusters were the hub genes we analysed. Similarly, the 23 genes that were downregulated in the cluster with the highest score included 20 hub genes that we found as a result of the PPI analysis (high vs cont).



Supplementary Figure 5. Top 2 modules from the PPI interaction networks **A.** Upregulated genes, **B.** Downregulated genes was analyzed by MCODE plugin in cytoscape software in Low vs control. Two clusters were selected for genes with high scores (MCODE score>3), representing upregulated genes, while a one cluster was chosen for downregulated genes, which is the most significant cluster included 20 hub genes that we found as a result of the PPI analysis (low vs control).