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Investigation of *ErbB* and Insulin Signaling Pathways in the Pathogenesis of Multiple Myeloma

Multipl Miyelom Patogenezinde ErbB ve İnsülin Sinyal Yolaklarının İncelenmesi

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Abstract

Aim: Analysis of genes that play roles in multiple myeloma pathogenesis and their pathways are current research topics. We aimed to detect expression of some genes in *ErbB* and insulin signaling pathways.

Methods: Bone marrow samples were taken from three healthy volunteers and 17 treatment-naive patients. Firstly RNA isolation was made and then cDNA were synthesized. There are eight genes that are related to *ErbB* and insulin signaling pathways. Specific primers for these genes were designed. Gene expression analysis was performed by the real-time polymerase chain reaction method.

Results: In the patient group, expressions of *MTOR, RPTOR, PIK3CA, AKT1, ErbB4, PRKAR2A* and *PRKACB* genes were detected to be 3-10 times up-regulated than in control group. There were no differences in the expression levels of *RICTOR* and *GYS1* genes between control group and patient group. *GYS1*, *PRKACB* and *PRKAR2A* genes have been analyzed for the first time.

Conclusion: In this study, *PRKAR2A* and *PRKACB* genes expressions were detected to be upregulated and this has not been reported in the literature before. *MTOR, RPTOR, PIK3CA, AKT1*, and *ErbB4* genes expression were detected to be upregulated as has been reported in the literature. All these results will be useful to understand the pathogenesis of multiple myeloma.

Keywords: Multiple myeloma, qReal-time-polymerase chain reaction, insulin signaling pathway, *ErbB*

Öz

Amaç: Multiple myeloma patogenezinde rol oynayan genlerin ve ilgili yolakların moleküler düzeyde incelenmesi güncel bir araştırma alanıdır. *ErbB* ve insülin sinyal yolağında bulunan bazı genlerin ekspresyon çalışmasının yapılması amaçlandı.

Yöntemler: Üç sağlıklı gönüllü ve 17 tedavisiz hastadan kemik iliği alındı, ilk olarak RNA izolasyonu yapıldı ve daha sonra cDNA sentezi yapıldı. *ErbB* ve insülin sinyal yolakları ile ilgili sekiz gen vardır, bu genler için spesifik primerler dizayn edilmiştir. Gen ekspresyon analizi, real-time polimeraz zincir reaksiyonu yöntemi ile gerçekleştirilmiştir.

Bulgular: Hasta grubunda *MTOR, RPTOR, PIK3CA, AKT1, ErbB4, PRKAR2A* ve *PRKACB* genlerinin ekspresyonu kontrol grubuna göre 3-10 kat arttı. Kontrol grubu ile hasta grubu arasında *RICTOR* ve *GYS1* genlerinin ekspresyon düzeylerinde farklılıklar gözlenmedi. *GYS1*, *PRKACB* ve *PRKAR2A* genleri ilk kez analiz edilmiştir.

Sonuç: Bu çalışmada, *PRKAR2A* ve *PRKACB* gen ekspresyonlarının arttığı saptanmış ve daha önce literatürde bildirilmemiştir. *MTOR, RPTOR, PIK3CA, AKT1, ErbB4* gen ekspresyonunun daha önce literatürde bildirildiği gibi ekspresyonlarının arttığı tespit edildi. Tüm bu sonuçlar multipl miyelomun patogenezini anlamak için faydalı olacaktır.

Anahtar Sözcükler: Multipl miyelom, qReal-time-polimeraz zincir reaksiyonu, insülin sinyal yolağı, *ErbB*

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Introduction

Multiple myeloma (MM) is a clonally B cell malignancy and is described by the accumulation of malignant plasma cells in the bone marrow, the presence of a monoclonal immunoglobulin in the serum and/or urine, lytic bone lesions, frequent anemia, and renal impairment (1-3). The progression of MM begins as monoclonal gammopathy of undetermined significance (MGUS), progresses to smoldering myeloma, and becomes eventually (symptomatic) myeloma (4,5). MM accounts for approximately 10% of hematological malignancies (6). MM predominantly affects 71% of patients diagnosed at age 65 years and over (7).

MM is still considered an incurable malignancy (8). MM is a heterogeneous disease with different clinical outcomes, chromosomal aberrations, and molecular characteristics. The cause of MM has not yet been identified. Further knowledge of the biological events underlying the development of MM is needed to determine new biomarkers. Interactions of MM cells especially with mesenchymal stromal cells and osteoclasts cause activation of multiple cellular signaling pathways on myeloma cells (*PI3K/AKT, JAK/STAT3, RAS/RAF/MAPK/ERK, NFκB)* which support their proliferation, survival, migration and even resistance to therapeutic agents (5,9).

We aimed to determine the intracellular pathways involved in the pathogenesis of the disease with changing expression of the identified genes in insulin signaling pathway and ErbB signaling pathway in MM patients.

Methods

Patients

In this study, we included 17 patients (11 males, 6 females) aged 51-74 years who was diagnosed with MM according to the International Myeloma Working Group diagnostic criteria and Durie-Salmon criteria. The study was approved by the local Ethics Committee of İstanbul Faculty of Medicine, İstanbul University (No: 2014/927), and all patients provided informed consent in accordance with the Declaration of Helsinki.

Real-Time Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated from bone marrow using the RNeasy Mini kit (Qiagen Venlo, Netherlands) and RNA samples were quantified using a NanoDrop® ND-2000 spectrophotometer. Total RNA was reverse transcribed into total cDNA with the cDNA Synthesis Kit (Thermo Fisher Scientific, Wilmington, Delaware, USA). Gene expression analysis was performed by quantitative reverse transcription (qRT)-polymerase chain reaction (PCR) (LightCycler 480 II, Roche, Germany).

The PCR reaction started with a denaturation step at 95°C for 10 minutes (1 cycle), followed by 45 cycles at 95°C for 15 seconds, 60°C for 60 seconds and 60°C for 1 second. Subsequently, a melting curve program was applied with continuous fluorescence measurement. A standard curve for genes templates was generated through four times dilution of PCR products and the β-actin gene was used as reference for normalization of the gene expression levels. Each reaction was performed in duplicate. Designed primers are shown in Table 1.

The relative gene expression (fold change) was measured with the comparative threshold cycle (Ct) method using β-actin as housekeeping gene and the $2^{-\Delta\Delta Ct}$ formula.

Statistical Analysis

In all statistical analyses, the SPSS version 13.0 was used. The threshold cycle (Ct) was determined for each sample. ΔCt indicated the difference in expression levels with the Ct value of the related gene and mean of housekeeping gene ($\Delta Ct = Ct_x$ gene $- Ct_{housekeeping}$), and ΔΔCt indicated the difference in the ΔCt value between treatment and control groups ($\Delta\Delta Ct = \Delta Ct_{ES} - \Delta Ct_{control}$). The p values were calculated based on a Student's t-test of the replicate $2^{-\Delta Ct}$ values for each gene in the control and treatment groups. All tests were two-sided, and a p value of less than 0.05 was considered statistically significant.

Table 1. Primer sequences

MTOR (F)	5'- CTAAGTCTACCACGACAGCCCGG-3'
MTOR (R)	5'- GGCCTTCATGCCACATCTCATGCC-3'
RICTOR (F)	5'- CAACTGGGATGCTGTGAGGCATAG-3'
RICTOR (R)	5'- GTACTAGTAGAGCTGCTGCCAAAC-3'
RAPTOR (F)	5'- GAGAAGCTCTACAGCCTCCTCTCC-3'
RAPTOR (R)	5'- CCGTCCTCTCTGCAGAGTTGC-3'
PIK3CA (F)	5'- ACTTATTGAGGTGGTGCGAAAT -3'
PIK3CA (R)	5'- TGATGTAGTGTGTGGCTGTTGA-3'
AKT1 (F)	5'- GGGTTTCTCCCAGGAGGTTT-3'
AKT1 (R)	5'- GTCCATGGTGTTCCTACCCA -3'
ErbB4 (F)	5'- AGGAGTGAAATTGGACACAGC-3'
ErbB4 (R)	5'- TCCATCTCGGTATACAAACTGGT-3'
PRKAR2A (F)	5'- CCTAGCAGATTTAATAGACG-3'
PRKAR2A (R)	5'- ATCATCTCCTTGGTCAATGA-3'
PRKACB (F)	5'- GTTCTTTCTACCAATCTATATGTCTTTC-3'
PRKACB (R)	5'- ATGGACCAGTGAAATCAATATC-3'
GYS1 (F)	5'- GCCTTTCCAGAGCACTTCAC-3'
GYS1 (R)	5'- CTCCTCGTCCTCATCGTAGC-3'
β-ACTIN (F)	5'- AGAGCTACGAGCTGCCTGAC -3'
β-ACTIN (R)	5'- AGCACTGTGTTGGCGTACAG -3'

Table 2. The average and standard deviation values of the clinical parameters of the patients

	Average	Standard deviation	Referenve intervals
Albumin (g/dL)	3.13	0.824	3.75-5.01
Creatin (mg/dL)	1.41	1.324	0.52-1.08
Hb (g/dL)	10.331	2.606	12.1-15.9
Hct (%)	30.77	6.85	-
White blood cell (x10^3/µL)	7.09	2.5	3.2-10.6
Red blood cell (x10^6/µL)	3.63	0.809	3.88-5.46
Platelet (x10^3/µL)	210.8	88.26	150000-440000
M spike (g/dL)	2.39	2.62	2.39
Kappa light chain (mg/L)	9876.7	11341.44	3.3-19.4
Lambda light chain (mg/L)	3006.55	4267.3	5.71-26.3
Age	62.76	7.51	-
Beta microglobulin (mg/L)	0.06	0.147	-

Hb: Hemoglobin, Hct: Hematocrit

Table 3. The expression levels of genes, confidence interval and p value

Genes	Fold regulation	95% CI	p value
ErbB4	10.5968	6.93-14.26	0.004517
MTOR	5.9518	3.00-8.91	0.013228
PRKAR2A	5.8204	1.39-10.25	0.006539
PRKACB	3.1383	1.57-4.71	0.029055
PIK3CA	3.3122	1.80-4.82	0.023204
AKT1	6.498	0.92-12.08	0.054141
RPTOR	9.6911	5.88-13.50	0.006912
RICTOR	1.1783	0.83-1.52	0.342337
GYS1	-1.3493	0.42-1.06	0.205086
β-ACTIN	1	1.00-1.00	0

CI: Confidence interval

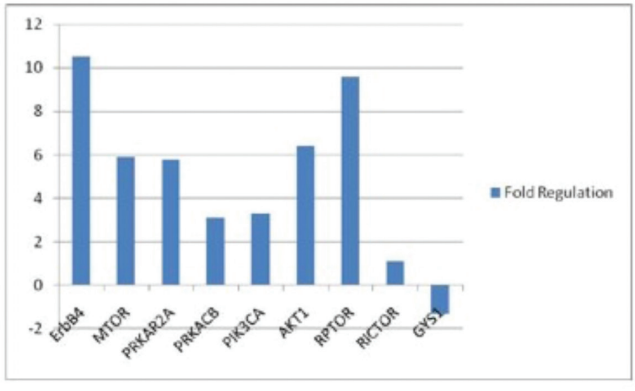

Figure 1. Graph showing fold change of genes

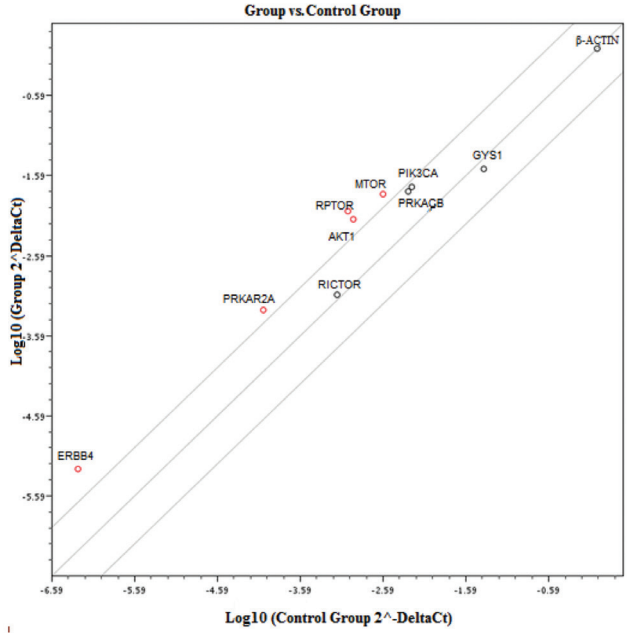

Figure 2. Distribution graph showing differences in expression level of genes

Results

The average and standard deviation values of the clinical parameters of the patients are shown below (Table 2). The expression levels of genes in the *ErbB* and insulin signaling pathways are shown in Figure 1, 2 and Table 3.

Discussion

Currently, development of novel targeted therapies for MM is a very active area of research. Advances in molecular biological thechniques and understanding the interactions between genes in pathways related to disease pathogenesis and prognosis are expected to allow the use of new targeted therapies in the near future. Signal transduction is now considered to be the one of the key mechanisms impaired in many types of cancer. Therefore, identifying the main pathways, the genes interacting with each other in these pathways and novel prognostic markers will help not only early diagnosis of MM, but also effective treatment of this disease.

In 2002, Sukru Ozturk et al. made a project named "Comparative Gene Expression Profilling of Multiple Myeloma, Smoldering Myeloma and Monoclonal Gammopathy Undetermine Significance Caces" and found 405 fusion sequences. This project was supported by İstanbul University (Project no: 7348). These fusions were analyzed using the UCSC website (https://genome.ucsc.edu/). After that, using Venny program, we found the genes that can be related with the disease. These genes were analysed by WebGestalt database and ten pathways were detected; because of financial means, we choosed two of them. Insulin

and *ErbB* signaling pathways, which take part especially in cell poliferation and protein synthesis, were evaluated by bioinformatics analysis.

ErbB4 is a transmembrane tyrosine kinase that regulates cell proliferation and differentiation (10). *ErbB4* does not arise from hematopoietic origin, but is known to be associated with poor prognosis in endometrial cancer (11). *ErbB4* gene expression levels decreases in pancreas and kidney tumors. In many studies, it has been shown that *ErbB4* was overexpressed in series of breast cancer, colorectal cancer and osteosarcoma (12). In their study, Mahtouk et al. (13) reported expression of *ErbB4* gene in 9 of 17 human myeloma cell lines. Also primary myeloma cells were expressed in 14 of 21 patients, and they showed that the gene was not expressed in normal plasmablastic cell and bone marrow plasma cells. In this study, *ErbB4* gene was found to be nine fold upregulated in the bone marrow of MM patients.

MTOR is a molecular sensor that regulates cell proliferation, protein synthesis, and promotes cell cycle progression from G_1 to S phase (14). Maiso and et al. (15) showed that MM1S, U266 and U266LR7 cell lines have low *RPTOR* gene expression; *RAPTOR* and *RICTOR* genes were expressed in OPM1, OPM2, MM1R, H929 and *RPM18226* cell lines. In our study, we did not find a significant expression of *RICTOR*; we found that *RAPTOR* and *MTOR* were upregulated nine fold and five fold, respectively.

The regulation of *PI3K/AKT/MTOR* pathway impaired in human cancers and this affects cell survival, proliferation and metastasis. Phosphatidylinositol are messenger molecule of lipid kinases that are a subclass of the *PI3Ks*. *PI3Ks* are activated by cell membrane receptors. When *PI3Ks* are phosphorylated, they act as second messenger activating downstream pathways including *AKT* (14). *PI3K/AKT* signaling pathway is involved in cell survival and proliferation in various cancers. Inhibition of *PI3K* leads to MM cell death but overactivation leads to cell proliferation (16). In their study performed in 2013, Azab et al. (17) in 2013, they detected increased gene expression of *PI3KCA* in MM. Our results are consistent with the literature; we showed that *PI3KCA* gene expression was upregulated three fold.

The most important down-stream effector of the *PI3K* pathway is a protein kinase B, also known as *AKT*. *AKT* is involved in cellular processes such as cell proliferation survival and migration, glucose metabolism and transcription of genes (18,19). In a study by López-Corral et al. (20), it has been showen that *AKT1* gene was more expressed in MM than in MGUS. In our study, we found that the expression of *AKT1* gene increased six times.

There was no statistically significant difference in the level of *GYS1* gene expression between patients and controls. It can be explained by two reasons; first *GYS1* gene encodes the rate-limiting enzyme for glycogen synthesis. This catalyst function is regulated through phosphorylation by kinases. Especially the expression levels of the gene change according to stage of the disease. Second, in cardiovascular disease, *GYS1* expression levels increase because *GYS1* gene is expressed mostly in skeletal and cardiac muscle.

PKA is acAMP-dependent protein kinase and takes part in *AKT* signal transduction pathway. *PKA* plays a role in antilipolytic mechanism, cell growth, apoptosis and gene transcription. Recently, it has been shown that the disruption in regulation of *PKA* causes dysregulation of D-type cyclins including cyclin D1. Schraders et al. (21) stated that the expression of *PRKACB* gene decreases in half of mantle cell lymphoma tumors. The levels of cAMP are so important because of their effects on cell cycle proliferation, apoptosis and cyclin D1.

It has been shown that *PKA* genes subtypes had different expression levels in many types of cancer. It is upregulated in stomach cancer and breast cancer (22,23). In our study, *PRKACB* gene was three fold upregulated also; there has been no other study analyzing the relationship between *PRKACB* gene level and MM.

AKT gene is induced by *PI3K* which is in insulin signal pathway and is phosphorylated by *PDK1/2*. *AKT* leads to protein synthesis by phosphorylating *MTOR* and *RAPTOR* genes and induces glycogenesis by dephosphorylation of *GYS* and *PHK* genes. The *RAS* gene which exists in the same pathway leads to differentiation and proliferation by phosphorylating *RAF, MEK* and *ERK1/2* genes. All these events suggest that all the genes are involved in different mechanism and they alter mRNA levels of each other. All these effects are correlated with and connected to each other. The results of our study showed that especially gene expression levels increase approximately in the same rate with each other in MM patients. The consistency of our findings supports their pathogenetic significance.

PKA which takes part in insulin signal pathway is active after phosphorylation. *PRKAR2A* and *PRKACB* are the most remarkable subtypes among the subtypes of proteins because the genes have both catalytic and regulatory functions. There are many studies showing that *PRKAR2A* gene is upregulated in cancer. For example; in their study performed in 2013, Bidkhori et al. (24) stated that *PRKAR2A* was overexpressed in lung adenocarcinoma. In their study with 29 patients performed in 2004, Neben et al. (25) found that *PRKAR2A* was related to centromere structure and functions. According to the cell growth results, *PRKAR2A* was expressed greater than two fold in AML patients. Our results are correlated with those of the study by Neben et al. (25) *PRKAR2A* expression increases in breast, colorectal and various human non-endocrine cancers (26) and especially in cervical cancer, and this increased expression is related with poor prognosis. In our study, we determined that *PRKAR2A* gene is five fold upregulated. To our knowledge, there is no study showing the relationship of this gene with MM.

Conclusion

In conclusion, insulin signaling pathway is involved in protein synthesis, gluconeogenesis and proliferation. *ErbB* signaling pathway is also involved in protein synthesis and cell cycle. In this study, some gene expressions which exist in insulin and *ErbB* signal pathways (*MTOR, RPTOR, PIK3CA, AKT1, ErbB4, PRKAR2A, and PRKACB*) were analyzed. There was no significant difference between the expression of *GYS1* and *RICTOR* genes in MM patients. Especially *ErbB4* and *MTOR, RPTOR, PIK3CA, AKT1, PRKAR2A, PRKACB* genes expression levels were found to be 3-10 times greater than in control group. Our results can be useful for explanation of the etiopathogenesis of MM.

Authorship Contributions

Surgical and Medical Practices: G.Ç., E.O., M.A., M.N.Y. Concept: Ş.Ö., D.Ü., Ş.P., K.C., E.M.C. Design: E.M.C., Ş.P., K.C. Data Collection or Processing: D.Ö., E.M.C., C.G.E. Analysis or Interpretation: E.M.C., D.Ö. Literature Search: D.Ö. Writing: D.Ö.

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References

1. Stella F, Pedrazzini E, Agazzoni M, et al. Cytogenetic Alterations in Multiple Myeloma: Prognostic Significance and the Choice of Frontline Therapy. Cancer Invest 2015;33:496-504.
2. Binsfeld M, Fostier K, Muller J, et al. Cellular immunotherapy in multiple myeloma: lessons from preclinical models. Biochim Biophys Acta 2014;1846:392-404.
3. Cirit M, Uzum A, Ozen P, et al. The value of serum immunoglobulin free light chain assessment in patients with monoclonal gammopathies and acute renal failure. Turk J Haematol 2012;29:385-91.
4. Fairfield H, Falank C, Avery L, et al. Multiple myeloma in the marrow: pathogenesis and treatments. Ann N Y Acad Sci 2016;1364:32-51.
5. Prideaux SM, Conway O'Brien E, Chevassut TJ. The genetic architecture of multiple myeloma. Adv Hematol 2014:864058.
6. Mikhael JR, Dingli D, Roy V, et al. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus guidelines 2013. Mayo Clin Proc 2013;88:360-76.
7. Andrews SW, Kabrah S, May JE, et al. Multiple myeloma: the bone marrow microenvironment and its relation to treatment. Br J Biomed Sci 2013;70:110-20.
8. Glavey SV, Manier S, Sacco A, et al. Epigenetics in Multiple Myeloma. Cancer Treat Res 2016;169:35-49.
9. Garcia-Gomez A, Sanchez-Guijo F, Del Cañizo MC, et al. Multiple myeloma mesenchymal stromal cells: Contribution to myeloma bone disease and therapeutics. World J Stem Cells 2014;6:322-43.
10. Ni CY, Murphy MP, Golde TE, et al. gamma -Secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. Science 2001;294:2179-81.
11. Saghir FS, Rose IM, Dali AZ, et al. Gene expression profiling and cancer-related pathways in type I endometrial carcinoma. Int J Gynecol Cancer 2010;20:724-31.
12. Paatero I, Elenius K. ErbB4 and its isoforms: patentable drug targets? Recent Pat DNA Gene Seq 2008;2:27-33.
13. Mahtouk K, Hose D, Rème T, et al. Expression of EGF-family receptors and amphiregulin in multiple myeloma. Amphiregulin is a growth factor for myeloma cells. Oncogene 2005;24:3512-24.
14. Rossi M, Di Martino MT, Morelli E, et al. Molecular targets for the treatment of multiple myeloma. Curr Cancer Drug Targets 2012;12:757-67.
15. Maiso P, Liu Y, Morgan B, et al. Defining the role of TORC1/2 in multiple myeloma. Blood 2011;118:6860-70.
16. Han K, Xu X, Chen G, et al. Identification of a promising PI3K inhibitor for the treatment of multiple myeloma through the structural optimization. J HematolOncol 2014;7:9.
17. Azab F, Vali S, Abraham J, et al. PI3KCA plays a major role in multiple myeloma and its inhibition with BYL719 decreases proliferation, synergizes with other therapies and overcomes stroma-induced resistance. Br J Haematol 2014;165:89-101.
18. Toker A, Yoeli-Lerner M. Akt Signaling and Cancer: Surviving but not Moving On. Cancer Res 2006;66:3963-6.
19. Cao H, Zhu K, Qiu L, et al. Critical role of AKT protein in myeloma-induced osteoclast formation and osteolysis. J Biol Chem 2013;288:30399-410.
20. López-Corral L, Corchete LA, Sarasquete ME, et al. Transcriptome analysis reveals molecular profiles associated with evolving steps of monoclonal gammopathies. Haematologica 2014;99:1365-72.
21. Schraders M, Jares P, Bea S, et al. Integrated genomic and expression profiling in mantle cell lymphoma: identification of gene-dosage regulated candidate genes. Br J Haematol 2008;143:210-21.
22. Furuta K, Arao T, Sakai K, et al. Integrated analysis of whole genome exon array and array-comparative genomic hybridization in gastric and colorectal cancer cells. Cancer Sci 2012;103:221-7.
23. Livshits A, Git A, Fuks G, et al. Pathway-based personalized analysis of breast cancer expression data. Mol Oncol 2015;9:1471-83.
24. Bidkhori G, Narimani Z, Hosseini Ashtiani S, et al. Reconstruction of an integrated genome-scale co-expression network reveals key modules involved in lung adenocarcinoma. PLoS One 2013;8:e67552.
25. Neben K, Tews B, Wrobel G, et al. Gene expression patterns in acute myeloid leukemia correlate with centrosome aberrations and numerical chromosome changes. Oncogene 2014;23(13):2379-84.
26. Vincent-Dejean C, Cazabat L, Groussin L, et al. Identification of a clinically homogenous subgroup of benign cortisol-secreting adrenocortical tumors characterized by alterations of the protein kinase A (PKA) subunits and high PKA activity. Eur J Endocrinol 2008;158:829-39.