Iran. J. Biotechnol. July 2021;19(3): e2643 DOI: 10.30498/ijb.2021.209370.2643

Research Article

Filtering of the Gene Signature as the Predictors of Cisplatin-Resistance in Ovarian Cancer

Atousa Ataei[1], Seyed Shahriar Arab[2*], Javad Zahiri[2], Azam Rajabpour[3], Konstantin Kletenkov[1], Albert Rizvanov[1*]

[1]Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia
[2]Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.
[3]Department of Molecular medicine, Pasteur Institute of Iran, Tehran, Iran

[*]*Corresponding author:* Seyed Shahriar Arab, Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran. Tel: +98- 2182883494, Fax: +98- 2182884717, E-mail: sh.arab@modares.ac.ir
[*]*Co-Corresponding author:* Albert Rizvanov, Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russia.Tel: +784-32934307, Fax: +784-32927418, E-mail: Albert.Rizvanov@kpfu.ru

Background: Gene expression profiling and prediction of drug responses based on the molecular signature indicate new molecular biomarkers which help to find the most effective drugs according to the tumor characteristics.

Objectives: In this study two independent datasets, GSE28646 and GSE15372 were subjected to meta-analysis based on Affymetrix microarrays.

Material and Methods: In-silico methods were used to determine differentially expressed genes (DEGs) in the previously reported sensitive and resistant A2780 cell lines to Cisplatin. Gene Fuzzy Scoring (GFS) and Principle Component Analysis (PCA) were then used to eliminate batch effects and reduce data dimension, respectively. Moreover, SVM method was performed to classify sensitive and resistant data samples. Furthermore, Wilcoxon Rank sum test was performed to determine DEGs. Following the selection of drug resistance markers, several networks including transcription factor-target regulatory network and miRNA-target network were constructed and Differential correlation analysis was performed on these networks.

Results: The trained SVM successfully classified sensitive and resistant data samples. Moreover, Performing DiffCorr analysis on the sensitive and resistant samples resulted in detection of 27 and 25 significant (with correlation $\geq|0.9|$) pairs of genes that respectively correspond to newly constructed correlations and loss of correlations in the resistant samples.

Conclusions: Our results indicated the functional genes and networks in Cisplatin resistance of ovarian cancer cells and support the importance of differential expression studies in ovarian cancer chemotherapeutic agent responsiveness.

Keywords: Cisplatin-resistance, Gene expression analysis, Ovarian neoplasm, Regulatory network

Abbreviations:

DEGs: Differentially Expressed Genes, GEO: Gene Expression Omnibus, GFS: Gene Fuzzy Scoring, SLBP: Stem-Loop Binding Protein

1. Background

Although chemotherapy is generally the primary line of treatment for ovarian cancer, it might not be satisfactory due to the intrinsic and/or acquired chemoresistance (1). Pharmacogenetics believe that drug resistance appears as a result of various cellular processes including epigenetic modifications, impaired DNA repair proteins, deregulation of apoptotic pathways, increase in autophagy and decrease in the intracellular drug concentrations induced by drug

transporters (2). Several researches have proposed that evaluation of metabolic pathways would help researchers identify resistant patients to chemotherapy (3). Due to the diversity of molecular mechanisms involved in drug resistance, high-throughput methods such as RNA/miRNA sequencing or SWATH mass spectrometry may result in smarter patient monitoring in clinical trials for the identification of resistance-related biomolecules.

Challenges in cancer chemotherapy have led to the development of predicting methods for assessing drug efficacy in cell lines or human samples. The study of gene expression in cancer cells found that the pattern of gene expression in drug-resistant cells is far different and this phenomenon is caused by an alteration in the expression level of multiple genes (4). Using computational analysis methods to predict the pattern of drug resistance based on the patient's molecular and clinical profiles can effectively improve the accuracy and quality of treatment.

Gene regulatory network analysis is essential to unveil potential interactions in high-throughput experiments. Here, we introduce some mRNAs and transcription factors and we tend to reveal their regulatory effect in Cisplatin resistance gene regulation. Previous studies showed that altered expression of mRNA in ovarian cancer cell lines may play important role in promoting Cisplatin resistance. Therefore, in the present study, we focus on the differential expression and differential correlation of the genes in two datasets obtained from the NCBI GEO.

As currently, only two datasets of Cisplatin resistance and sensitive profiles are available in the Gene Expression Omnibus, we subjected meta-analyzing these gene expression profiles in Cisplatin-resistant and sensitive ovarian cancer cell lines that used similar array technology platforms.

Our further aim was to investigate transcription factor-gene, miRNA-gene and lncRNA-gene networks to present a complex network of genes and their regulators in Cisplatin resistance as well as to predict chemo-response in ovarian cancer.

2. Objectives

In this study, transcription factor-gene, miRNA-gene and lncRNA-gene networks and their regulators in Cisplatin resistance as well as chemo-response in ovarian cancer will be evaluated by computational analysis methods.

3. Materials and Methods

3.1. Datasets

Two independent microarray datasets including GSE28646 (5) and GSE15372 (6) were retrieved from the NCBI Gene Expression Omnibus (GEO) database (**Table 1**). GSE28646 is a part of the GSE28648 super series and includes 15 (A2780 cell line) samples, from which, we selected three Cisplatin-resistant and three Cisplatin sensitive samples for downstream analysis. GSE15372 is also a part of the GSE15709 super series and includes 10 (A2780 cell line) samples consisting of five Cisplatin-resistant and five Cisplatin sensitive samples.

3.2. Data Preprocessing and Reconnaissance of Differentially Expressed Genes

The recently introduced Gene Fuzzy Scoring (GFS) method was employed to remove data batch effects as well as to normalize the data between 0 and 1. GFS is

Table 1. Detailed information of used datasets

Micro array Datasets	Platform	Description
GSE28646	GPL570 [HGU133_Plus_2]	Ovarian Cisplatin sensitive cell line A2780 (3 replicates) Ovarian Cisplatin resistant cell line CP70 (3 replicates) CP70 is a resistant primary cancer cell type derived from A2780.
GSE15372	GPL570 [HGU133_Plus_2]	Parental A2780 cells (Cisplatin sensitive) (GI50=5uM) Round5 (multiple treatment cycles) A2780 cells (Cisplatin resistant) (GI50=35uM)

a sensitive method which is capable of removing batch effects without introducing false positives. Using the GFS method, each expression is mapped to a number between 0 and 1 according to its rank in the sample sorted expression values (For more details please see (7)). Furthermore, the principal component analysis (PCA) was used to eliminate outlier data.

In order to detect the Differentially Expressed Genes (DEGs) in the sensitive/resistant samples, Wilcoxon Rank Sum test (aka Mann-Whitney U Test) was applied. Genes with the lowest p-value were selected as DEGs for further analyses. The reason for using the nonparametric Rank Sum test instead of t-test was the failure of the normality test in the t-test. This can be due to either the low number of samples or the gene fuzzy scoring (8). However, the Rank Sum test sufferes a drawback as it's p-value does not account for the amount of difference in expression values. Hence, we checked the genes with the lowest p-value and a fold change of at least two. For the down regulated genes the adjusted p-value was considered to be 0.00451 and for the up regulated genes the adjusted p-value was considered to be 0.000625. Moreover, log2Fc for the down regulated genes was -31.33495 and for the up regulated genes was 26.728436. Furthermore to validate the results, LIMMA package was also used to obtain DEGs.

3.3. Resistance Prediction Method

A simple Support Vector Machine (SVM) (9, 10) was employed to predict the resistance status of each sample. To this end, a t-test was performed on the resistance and sensitive samples and the first *n* DEGs were extracted as the features for SVM training. Jackknife re-sampling was then used for prediction evaluation.

3.4. Construction of Transcription factor-Target Regulatory Network

Transcription factors corresponding to the extracted DEGs were harvested from the Trrust database (11) and TF-target network was constructed using Cytoscape 3.4.

3.5. miRNA-Target Regulatory Network

Deregulated miRNAs in ovarian cancer Cisplatin resistance were harvested from the MirCancer 2017 database (12). The results were subsequently introduced into mirDB (13) to specify the corresponding miRNAs targets. The identified DEGs (both up and down-regulated genes) were then compared with the obtained miRNA-Target interactions in the previous step. Inverse patterns of deregulation between miRNAs and their targets, were selected to create of the miRNA-target regulatory network. This network was also visualized in Cytoscape. Moreover, MirCancer database was also searched for those deregulated miRNAs involved in chemoresistance development in different types of cancers. These miRNAs were similarly imported into mirDB to specify their corresponding target genes. The resultant miRNA-target interactions were filtered for those targets which are present in the list of identified up and down-regulated DEGs. The resulted interactions which show the converse pattern of expression were also visualized in Cytoscape.

To validate the extracted miRNA-mRNA interactions, starBase database (14) was applied. The 89 candidate chemoresistance genes were used as targets and miRNA interactions which were identified by 5 or more gene-target prediction programs in the starBase database were harvested. Harvested interactions were then analyzed using Cytoscape for degree and betweenness centralities. Nodes with degree centrality more than 10 and betweenness centrality more than 0.00001 were then fed into mirPath v.3 (15) and ToppGene suite (16) to reveal their corresponding pathways.

3.6. Construction of LncRNA-target Network

To reconstruct the corresponding lncRNA-target network of the 89 DEGs, lncTar (14) was used. The nucleotide sequences of 89 DEGs (considering all alternative spliced variants) as well as the sequences of all differentially expressed lncRNAs were collected in the FASTA format and were introduced into lncTar as inputs.

3.7. Differential Co-expression Analysis

The newly generated positive or negative correlations in resistant samples can shed light on the intricate deregulations which cannot be detected using differential expression analysis. Here, to find gene pairs which expression correlations have been changed between sensitive and resistant samples, *"comp.2.cc.fdr"* function of Diffcorr package (17) in R was used on the normalized, pre-processed data.

4. Results

4.1. Findings Genes Expression Patterns in Sensitive and Resistant Cell Lines

PCA scatter plot of the first three principal components of original raw data reveals presence of batch effect

in the data (**Fig. 1**). After applying the GFS method, batch effect was successfully removed in PC3 and samples could then be clustered according to the biological replicates.

To evaluate the SVM, Jackknife resampling was performed. In each iteration, one sample was removed and the SVM was trained using the remaining ones. The trained SVM was then tested using the removed sample. The procedure was repeated for every sample. The SVM successfully predicted the correct status of samples for all samples. Heatmap representation of down and up-regulated genes is shown in **Figure 2**.

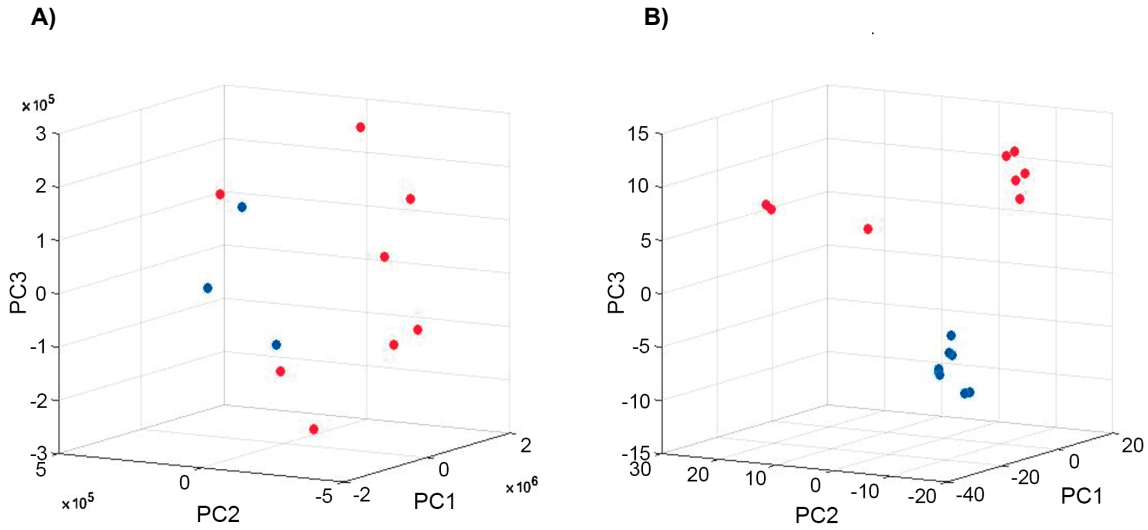

Figure 1. PCA scatter plot of the first three principal components of data before **A)** and after **B)** normalization. The colors blue and red correspond to cisplatin sensitive and cisplatin-resistant samples, respectively. Some sensitive samples in **A)** overlap with other samples

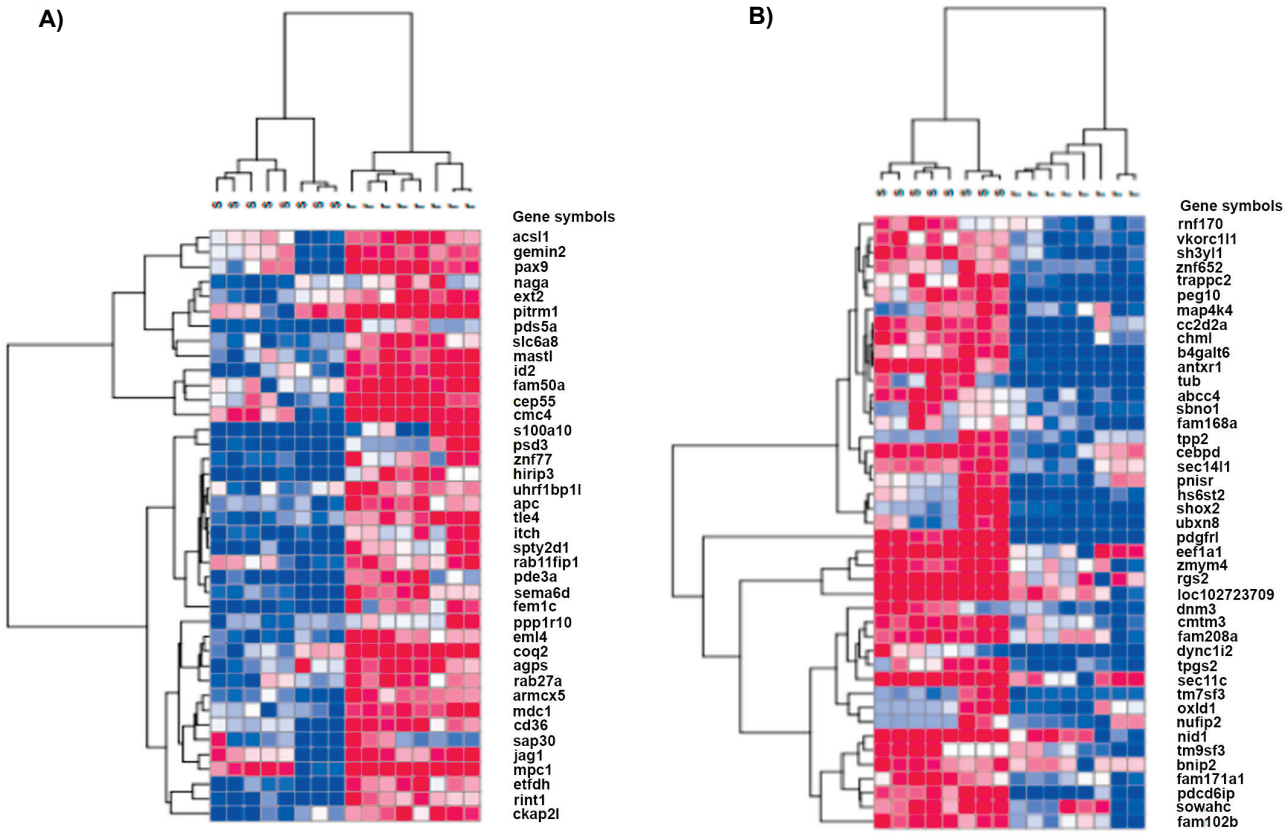

Figure 2. Heatmap representation of **A)** up-regulated and **B)** down-regulated genes

Both heatmaps show complete discrimination between sensitive and resistant samples.

4.2. Most of the Identified DEGs are Involved in the Resistance-associated Pathways

As was previously stated in the method section, DEGs were extracted by Wilcoxon Rank Sum test (**Table S1**). **Figure S1** represents the deregulated genes from **Table S1** which are associated with the process of tumor progression and chemotherapy resistance. Most of these genes are related to apoptosis, PI3k-AKT, MAPK, WNT and NOTCH signaling pathways. However, some are yet to be characterized. Furtheremore, to validate the results Limma package was also used to obtain DEGs. 62 out of 89 DEGs identified by the Wilcoxon rank sum test were also identified by the LIMMA package.

4.3. TF-target, miRNA-Target and The lncRNA-Target Network Analysis of Differentially Expressed Genes

TF-target regulatory network of deregulated genes is represented in **Figure 3A.** miRNA-target regulatory relationships between up and down-regulated miRNAs in ovarian cancer (blue and orange respectively) and their down- and up-regulated targets (pink and olive) has been shown in **Figure 3B** and **Figure 3C**.

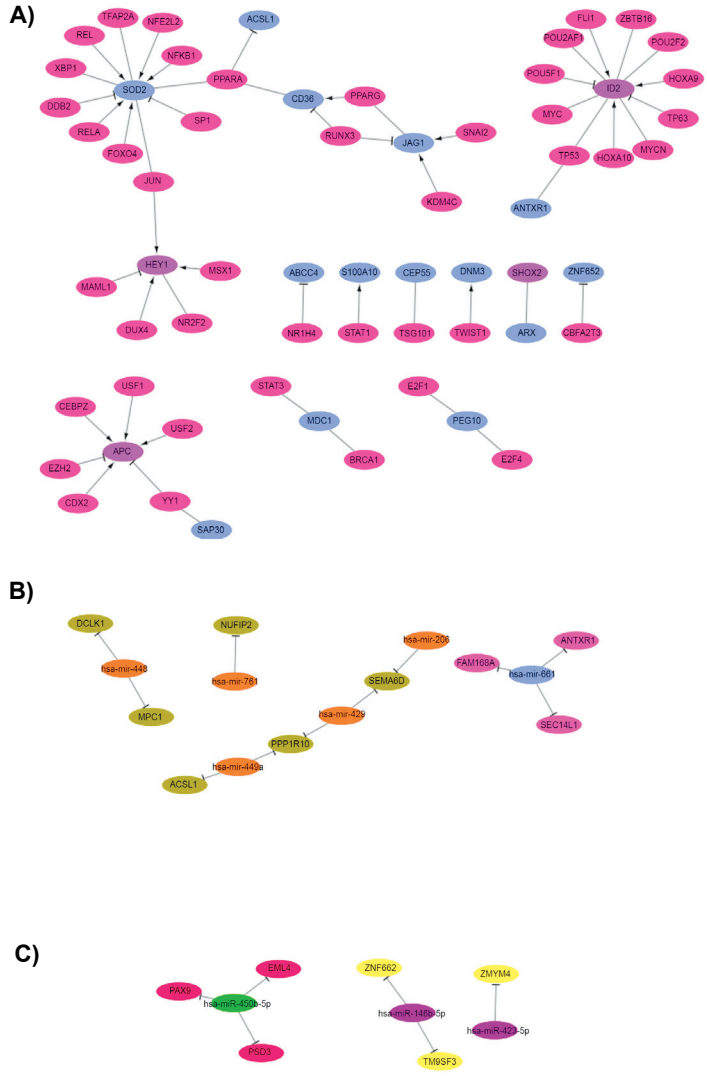

Figure 3. A: TF (pink-target regulatory network for up (violet) and down–regulated (blue) genes. Arrows, T bars, and solid lines show respectively activation, inhibition and unknown regulation of TFs on the target genes.

B, C: miRNA-target regulatory networks for deregulated miRNAs (blue and orange for respectively up and down-regulated miRNAs, pink and olive for down and up-regulated targets) previously reported in ovarian cancer **(B)** and those (green for down and violet for up-regulated miRNAs, red and yellow for respectively up and down-regulated targets) already known in different chemoresistance development in different cancers **(C)**

The regulatory relationships between deregulated miRNAs in chemoresistance (violet for the ups and green for the downs) and their corresponding targets in the list of down (yellow) and up-regulated (red) genes have been depicted.

The lncRNA-target interactions inferred from lncTar is shown in **Table S2**. Interactions with dGn (normalized dg) ≤ -0.15 was considered as significant. The lncRNA PLAC2 was the only lncRNA showing stable interactions with few transcripts.

To improve our results, starBase database was also used to extract miRNA-mRNA interactions. miRNAs with degree centrality more than 10 are enriched in pathways including ECM-receptor interaction, Adherens junction, cancer raletrd proteoglycans, Hippo signaling cascade, Fatty acid degradation, ErbB signaling pathway, Ubiquitin mediated proteolysis, Gap junction and genes with degree centrality more than 10 are enriched in pathways including Notch signaling pathway, Lactosylceramide biosynthesis, Endocytosis, Th1 and Th2 cell differentiation, TNF signaling pathway, Breast cancer, Signaling by NOTCH1 and NOTCH2 and Angiogenesis. Furthermore, nodes with betweenness centrality more than 0.00001 are enriched in pathways including Proteoglycans in cancer, Hippo signaling pathway, TGF-beta signaling pathway, Renal Cell Carcinoma, Adherens junction, Cell cycle, AMPK signaling pathway, FoxO signaling pathway, mTOR signaling pathway, Signaling pathway regulating pluripotency of stem cells, pathways in cancer, Transcriptional misregulation in cancer, Focal adhesion, p53 signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway and Wnt signaling pathway.

4.4. Identification of Differentially Correlated Genes in Sensitive and Resistant Cells

Performing DiffCorr analysis on the sensitive and resistant samples resulted into the detection of 27 and 25 significant (with correlation ≥|0.9|) pairs of genes that respectively correspond to newly constructed correlations and loss of correlations in the resistant samples (**Table S3**). Some of those genes which are involved in significant pathways including WNT, Adherens junction, CAMP, PI3K-AKT, JAK-STAT, MAPK, Calcium, Hedgehog, Notch, HIF-1, P53 and Cell Cycle are illistrated in **Figure S2**. Mechanisms of action for most of these genes are not yet determined. Detailed Diffcorr analysis results as well as correlation study of the genes and their corresponding pathway analysis are shown in **Table S4.**

4. Discussion

Resistance to chemotherapeutic agents is one of the most important obstacles in ovarian cancer. Cisplatin is a conventional chemotherapy agent that is used in the treatment of most solid tumors. Studies have shown that Cisplatin resistance is a multifaceted phenomenon that is affected by cellular events such as increased drug inactivation/efflux, defected DNA repair, alterations in drug toxicity and change in apoptotic processes (4). Microarray-based studies in ovarian cancer, illustrate a correlation between drug activity and shifts in the expression level of certain genes (4,18). Significant heterogeneity in drug response also have been expected due to the different pattern of gene expression in each cancer cell, even in cancer cells derived from the same cancer (19).

By analyzing microarray results, We have found 89 genes with a significant change including increased and decreased expression levels in the 8 Cisplatin-resistant samples. Twenty-five of the genes we reported in **Table S2** were not previously mentioned in the resistance of any type of cancer, including ovarian cancer. Though changes in expression levels of these genes indicate that these differences might be related to cellular responses to chemotherapy. However, confirmation of up/down regulation of these genes at the protein level in animal models and ovarian cancer patients before and after chemotherapy can provide evidence to accept or dismiss the possibility of involvement of these genes in response to the treatment.

Although our further investigation in pathway analysis showed a complex interrelationship between some of these genes, their regulating transcription factors, miRNA and lncRNA regulatory network and differential expression correlation exhibited that these genes are independently implicated in anti-cancer drug resistance, recurrence, and EMT.

One of the up-regulated genes was the exchange factor for ARF6R (EFA6) which has been previously identified as an ovarian cancer biomarker. Dietmar Pils et al. showed that EFA6 expression level is significantly lower in high grade tumor cells compared to normal ovarian samples or low grade tumors (20). A correlation between EFA6R expression and malignancies such as ovarian cancer has been reported, though extra experiments determining the role of EFA6R in ovarian cancer are essential (21).

Another up-regulated gene, doublecortin like kinase 1 (Dclk1), is a protein involved in the microtubule polymerization and stimulated microtubule elongation processes (22). Recently, it has been reported that

vital cellular signaling pathways associated with EMT and CSC such as NOTCH, NF-KB, and WNT (23) are governed by Dclk1 expression in cancer models (24). Dclk1 expressing cells are quiescent and are involved in cancer incidence, development, and invasion in pancreatic cancer. This kinase has been reported to be overexpressed in pancreas, liver, colon, esophagus, and intestines cancers and is known as a TSC marker. In gut cancers, the cell population with dclk1 overexpression also exhibits an enhanced expression of other TSC markers, including CD133, CD24 / CD44 / ESA, and ALDH (24). C. Benedikt Westphalen et al. have revealed that Dclk1 can bind to and operate with the oncogene, K-ras. According to their investigation, targeting of Dclk1 in K-ras-related cancers is an effective therapy method while therapeutic strategies targeting K-ras have failed in most of the trails (22). Other studies by Hui Wang et al. proved that there is a positive correlation between Dclk1 expression and overall survival (OS) in malignant pleural mesothelioma patients. They showed that the Dclk1 is regulated by MET/ERK5 signaling in human mesothelioma and inhibition of ERK5 and Dclk1 results in suppression of downstream oncogenic signalings like EMT, angiogenesis, pluripotency and anti-apoptotic which activity lead to chemoresistance. They suggested that MET/ERK5/Dclk1 signaling cascade could be a promising therapeutic target against mesothelioma (22). Other results demonstrated that Dclk-1 plays a regulatory role in cancer via miRNA dependent mechanisms. These studies showed that Dclk-1 adjusts the performance of Notch-1 in a post-transcriptionally manner, and Notch-1 itself is the downstream target of the miR-144. (25).

In another study, it was shown that suprresion of Dclk1 expression leads to an up-regulation of the miRNA let-7a, a negative modulator of cell cycle, and also to down-regulation of the oncogene c-myc. Thus a role in the acquisition of malignant properties can be considered for Dclk1 protein. Moreover, siRNA-mediated Dclk1 knockdown resulted in augmented expression of miR-200a that is an EMT suppresor and also in down-regulation of ZEB1 and ZEB2 with the subsequent rescue of E-cadherin in both human pancreatic and colorectal cancer cells (26). Recent studies revealed that Dclk1 play a role in the regulation of angiogenic factors (VEGFR1 and VEGFR2), downstream targets of miR-200a-c (27). Moreover, the knockdown of Dclk1 provoked the activation of miR-143/145 tumor suppressor miRNA cluster (22). These data suggest that Dclk1 is a regulator for the activation of ATM-mediated DNA damage response in tuft cells leading to the development of self-renewal/survival and drug-resistance properties in gut epithelial cells (**Fig. 4**) (23).

One of the down-regulated genes was zinc finger myelo-proliferative and mental retardation-type 4 (ZMYM4), a chromatin-interacting protein. ZMYM4 stimulates DNA repair through homologous recombination (HR) system (28). By performing a series of statistical hypothesis testing, Fang-Han Hsu and co-workers showed that 112 genes including ZMYM4 correlate with progression-free survival in patients who were only treated with Paclitaxel/Carboplatin after the surgery. Their results suggest that the region of this gene is associated with chemotherapy response (29).

Unlike our results, several studies stated the upregulation of PEG10 levels in hepatocellular carcinoma (HCC).

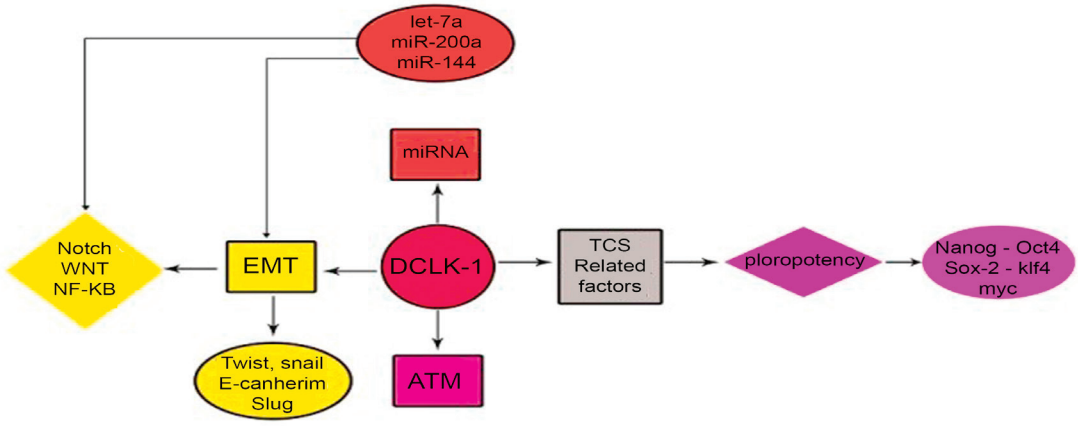

Figure 4. The molecular mechanisms involved in ovarian cancer resistance to cisplatin is associated with the Dclk- 1 overexpression in following features: 1) activation of EMT 2) microRNA profile 3) TSCs related factors like pluripotency 4) ATM involvement.

The data obtained from these studies Suggests PEG10 has a role in resistance to apoptosis induced by serum starvation and chemo-therapeutic drugs, and also stimulation of cell growth. PEG10 gene is located in an imprinted domain on human chromosome 7q21 and is characterized as a paternally expressed / maternally silenced gene. Although the mechanism of PEG10 regulation has not yet been elucidated, it has been shown to act as an apoptosis inhibitor in serum deprivation conditions and the presence of chemotherapy drugs. (30).

Qimin Zitan et al. reported that CHML is a cell growth suppressor agent which can cause a greater suppression effect in tumor cells than noncancerous cells. Their results suggested that CHML can trigger the activation of p53, contributing to CHML-induced apoptosis in some tumor cells such as MCF-7, RKO, and ML-I. They concluded that CHML prompts apoptosis through both the p53-dependent and independent cascades (31). According to their results and other investigations, it can be proved that down-regulated expression level of CHML in osteosarcoma cell lines and clinical samples, large diffuse B cell lymphoma and breast cancer is associated with poor survival (32).

In the survey of PM Neilsen and his colleagues, it was reported that decreased expression of ZNF652 was associated with breast cancer invasion and metastasis. They indicated that the down-regulation of ZNF652, a novel zinc-finger transcriptional repressor, induced by miR-155 causes up-regulation of TGFB1, TGFB2, TGFBR2, EGFR, SMAD2 and VIM as downstream targets (33).

FEM1C belongs to an evolutionarily-conserved VHL-box protein family that are all members of substrate recognition subunits of CUL2-RING E3 ubiquitin ligase complexes. FEM1 proteins regulate the Stem-Loop Binding Protein (SLBP) that is a conserved protein interacting with the stem-loop structure located in the 3′ end of canonical histone mRNAs and plays a role in mRNA cleavage and degradation mainly in the S-phase where histone synthesis occurs. It seems that the up-regulation of FEM1 results in SLBP enhancement and cellular degradation (34).

In parallel with our report, in the study of Chie Suzuki et al. it was reported that overexpression of Strawberry Notch Homolog 1) SBNO1(as a downstream component of the oncogenic Notch signaling pathway is associated with the proliferation of lung cancer cells (35, 36).

Protein phosphatase-1 nuclear targeting subunit PNUTS (PPP1R10) is a PTEN regulatory protein and it's downregulation is associated with increased apoptosis and decreased cellular proliferation in a PTEN-dependent manner (37).

The results of our study also showed that different molecular mechanisms are involved in the development of resistance to different chemotherapy drugs and therefore, we suggest a combinatory chemotherapy sterategy for cancer treatment.

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) analysis revealed that up-regulated DEGs mainly participated in the extracellular matrix and down-regulated DEGs were involved in the extracellular regions, differentiation of epithelial cells, and digestion. Furthermore, the KEGG pathways corresponding to the up-regulated DEGs included focal adhesion, ECM-receptor interaction, PI3K-Akt signaling pathway, PPAR signaling pathway, TGF-beta signaling pathway, Notch signaling pathway, MAPK signaling pathway, protein digestion, and absorption. While the down-regulated DEGs were enriched in nucleic acid binding, protein complex binding and microtubule cytoskeleton assembly proteins.

In addition to the identification of some transcription factor genes like ID2, HEY1 and APC with a major contribution to Cisplatin resistance amongst our DEGs, we looked for transcription factors which regulate our other differentially expressed genes. Interestingly, down-regulated transcription factor ID2 network including MYC family members and TP53 - which were previously reported to affect cell proliferation and also to amplify c-myc - and functional p53 lead to successful 5FU-based therapy in colorectal cancer patients (38, 39). Loss of function of transcription factor APC is reported in resistance to Cisplatin or doxorubicin-induced apoptosis through STAT3 activation (40). YY1 is a transcription factor that functions in biological processes including differentiation, development, and initiation of tumor and apoptosis (41). It was reported that inhibition of the YY1 leads to the sensitization of tumor cells to TRAIL-mediated apoptosis and therefore, YY1 can be used as an antitumor agent (42).

NF-κB which is a key transcription factor composed of homo or heterodimers from a pool of five REL proteins, is involved in the development, progression and chemoresistance of cancer by activating a number of mediators, including anti-apoptotic genes (43). Moreover, FOXO4 which is a member of FOXO transcription factors plays important roles in the regulation of cell fate by promoting cell survival and resistance to environmental stress (44). Although the exact mechanism by which FOXO family members

respond to chemotherapy is unclear, it is demonstrated that FOXO4 functions in downstream of the PI3-K signaling pathway and is a direct phosphorylation target for the protein kinase Akt (45). Another member of this network is endothelium-derived Jagged1 which is shown to promotes Notch activation, invasion, and chemoresistance to paclitaxel in B cell lymphoma (46). In our transcription factor network construction, paternally expressed gene 10 (PEG10) is regulated by E2F-1 which was previously reported to be involved in drug resistance through binding to PEG10 promoter (47).

Also, we showed that MDC1 is regulated by the STAT3 and BRCA1. MDC1 modulates the DNA damage pathway by STAT3 at the transcriptional level and overexpression of STAT3 leads to DNA repair and chemo-resistance. Also, it has been reported that MDC1 is involved in the recruitment of many DNA damage response proteins such as 53BP1 and the Mre11/Rad50/NBS1 (MRN) complex to the damage sites (48), (49).

One important factor in the field of pharmacogenetics contributes to microRNAs (miRNAs) as the members of the small non-coding RNAs, which are known as the post-transcriptional regulators. miRNAs bind to their complementary target messenger RNAs (mRNAs) leading to the translational repression or degradation of target mRNA (4). Recent reports have proved the critical roles for miRNAs in the development of drug resistance by regulation of hundreds or thousands of genes (5). It seems that analyzing the miRNA-gene regulatory network is essential to unveil the probable interactions in high-throughput experiments and computational methodologies. Here, our study found a signature of previously reported 9 deregulated miRNAs in ovarian cancer Cisplatin resistance which are associated with our DEGs. However, experimental assays in the approvement of the target-miRNA relation could provide a better understanding of these complex networks.

Differentially expressed lncRNAs annotated with transcripts of our DEGs demonstrated that PLAC2 is an lncRNA targeting the PITRM1 transcript variant 1, 2, 4, 5, 9, 6 and 3, PDS5A transcript variant 1 and MPC1 transcript variant 1, 2. As it is shown in **Table S3** except PITRM1, the other genes are related to chemoresistance. It was reported that cancer progression as well as mRNA stabilization of the genes involved in differentiation of epidermal tissue are associated with regulation of the staufen-1 protein-mediated by lncRNA PLAC2 transcript (50). However, in another study, cytoplasmic lncRNA PLAC2 expression was shown to be involved in cell cycle arrest in glioma through binding to the signal transducer and activator of transcription (STAT) 1 and inhibition of STAT1 nuclear transfer. Since the promoter of the ribosomal protein RPL36 (as the cell proliferation promoter and G1/S cell cycle progression factor) interacts with STAT1, decreased levels of cytoplasmic STAT1 can induce cell cycle inhibition in glioma (51). Further *in vivo* and *in vitro* experiments on this lncRNA and its target genes may pave the way for finding their precise mechanism of action as well as potential individual therapies.

In our regulatory network analyses of the Cisplatin-resistant ovarian cancer cell line, we administered the Differential Correlation analysis (DiffCorr) (DCG) which uses Fisher's z-test to test differential correlation between two groups (52). These differentially associated groups indicated potential biological function in Cisplatin-resistant A2780 cells in comparison with the sensitive cells. By integrating the enrichment analyses into our results, we identified several genes with differential coexpression in Cisplatin resistant cells compared to sensitive cell lines involved in the cAMP signaling pathway, HIF-1 signaling pathway, FoxO signaling pathway, Cell cycle, Wnt signaling pathway, Notch signaling pathway, TGF-beta, PI3K-Akt signaling pathway. An example of such differential correlation was ATF2 which has been found to play role in MAPK signaling pathway, PI3K-Akt signaling pathway and TNF signaling pathway which are all involved in drug resistance (**Table S4**). We also observed extensive metabolic pathways in differentially co-expressed genes. Pathways such as mRNA surveillance pathway, RNA degradation ubiquitin-mediated proteolysis and protein processing in endoplasmic reticulum exhibited significant changes in expression correlation between sensitive and resistant cell lines. Moreover, comparative analyses of differentially co-expressed genes and differentially expressed genes showed that only TPP2 is both DCG and DEG indicating that it has different coexpression partners under different conditions and is also differentially expressed. TPP2 is a serine peptidase that is localized in the centrosome. It has been reported that TPP2 overexpression leads to centriole multiplication and is implicated in c-MYC-associated Burkitt lymphoma cells (53,54). This observation highlighted the importance of DCGs analyses in resistance-related genes. Also, identifying differentially co-expressed genes in expression networks would help to detect regulatory genes involved in phenotype alterations such as resistance

induction. Taken together, the integration of high-throughput experimental data with computational analysis would help to identify the exact gene interactions in specific phenotype.

5. Conclusion

In this paper, we detected genes with altered expression levels in cisplatin-resistant ovarian cancer cells. To date, most drug resistance studies in ovarian cancer have performed concentrating on the proposed gene networks, which address a limited number of chemo-resistance-related genes in other types of cancer. In contrast, data obtained from microarray technology, as well as appropriate tools for identifying expressed/coexpressed genes in cisplatin-resistant ovarian cancer tissues, afford valuable information about potential new genes associated with drug resistance. This information can be applied for the improvement of strategies for cancer and may be conducted to more accurate personalized therapy.

Acknowledgments

This work was funded by the subsidy allocated to KFU for the state assignment 0671-2020-0058 in the sphere of scientific activities. This work is part of Kazan Federal University Strategic Academic Leadership Program.

References

1. Dressman HK, Berchuck A, Chan G, Zhai J, Bild A, Sayer R, *et al.* An integrated genomic-based approach to individualized treatment of patients with advanced-stage ovarian cancer. *Am J Clin Oncol.* 2007;**25**(5):517-525. doi : 10.1200/JCO.2006.06.3743
2. Shen D-W, Pouliot LM, Hall MD, Gottesman MM. Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. *Pharmacol Rev.* 2012;**64**(3):706-721. doi: 10.1124/pr.111.005637
3. Abad E, García-Mayea Y, Mir C, Sebastian D, Zorzano A, Potesil D, *et al.* Common metabolic pathways implicated in resistance to chemotherapy point to a key mitochondrial role in breast cancer. *Mol Cell Proteom*. 2019;**18**(2):231-244. doi: 10.1074/mcp.RA118.001102
4. Sherman-Baust CA, Becker KG, Wood III WH, Zhang Y, Morin PJ. Gene expression and pathway analysis of ovarian cancer cells selected for resistance to cisplatin, paclitaxel, or doxorubicin. *J Ovarian Res*. 2011;**4**(1):21. doi: 10.1186/1757-2215-4-21
5. Zeller C, Dai W, Steele NL, Siddiq A, Walley AJ, Wilhelm-Benartzi C, *et al.* Candidate DNA methylation drivers of acquired cisplatin resistance in ovarian cancer identified by methylome and expression profiling. *Oncogene.* 2012;**31**(42):4567. doi: 10.1038/onc.2011.611
6. Li M, Balch C, Montgomery JS, Jeong M, Chung JH, Yan P, *et al.* Integrated analysis of DNA methylation and gene expression reveals specific signaling pathways associated with platinum resistance in ovarian cancer. *BMC medical genomics.* 2009;**2**(1):34. doi: 10.1186/1755-8794-2-34.
7. Belorkar A, Wong L. GFS: fuzzy preprocessing for effective gene expression analysis. *BMC bioinformatics.* 2016;**17**(17):540. doi:10.1186/s12859-016-1327-8
8. Kim H-Y. Statistical notes for clinical researchers: Nonparametric statistical methods: 1. Nonparametric methods for comparing two groups. *Restor Dent Endod*. 2014;**39**(3):235. doi: 10.5395/rde.2014.39.3.235
9. Huang S, Cai N, Pacheco PP, Narrandes S, Wang Y, Xu W. Applications of support vector machine (SVM) learning in cancer genomics. *Cancer Genom Proteom.* 2018;**15**(1):41-51. doi: 10.21873/cgp.20063.
10. Noble WS. What is a support vector machine? *Nat. Biotechnol.* 2006;**24**(12):1565-1567. doi: 10.1038/nbt1206-1565.
11. Han H, Cho J-W, Lee S, Yun A, Kim H, Bae D, *et al.* TRUST v2: an expanded reference database of human and mouse transcriptional regulatory interactions. *Nucleic Acids Res*. 2018;**46**(D1):D380-D386. doi: 10.1093/nar/gkx1013
12. Xie B, Ding Q, Han H, Wu D. miRCancer: a microRNA–cancer association database constructed by text mining on literature. *Bioinformatics.* 2013;**29**(5):638-644. doi: 10.1093/bioinformatics/btt014
13. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Res.* 2020;**48**(D1):D127-D131. doi: 10.1261/rna.965408
14. Li J-H, Liu S, Zhou H, Qu L-H, Yang J-H. starBase v2. 0: decoding miRNA-ceRNA, miRNA-ncRNA and protein–RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res.* 2014;**42**(D1):D92-D97. doi: 10.1093/nar/gkt1248
15. Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, *et al*. DIANA-miRPath v3. 0: deciphering microRNA function with experimental support. *Nucleic Acids Res*. 2015;**43**(W1):W460-W466. doi: 10.1093/nar/gkv403
16. Chen J, Bardes EE, Aronow BJ, Jegga AG. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic Acids Res.* 2009;**37**(suppl_2):W305-W311. doi: 10.1093/nar/gkp427
17. Fukushima A. DiffCorr: an R package to analyze and visualize differential correlations in biological networks. *Gene.* 2013;**518**(1):209-214. doi: 10.1016/j.gene.2012.11.028
18. Duan Z, Feller AJ, Penson RT, Chabner BA, Seiden MV. Discovery of differentially expressed genes associated with paclitaxel resistance using cDNA array technology: analysis of interleukin (IL) 6, IL-8, and monocyte chemotactic protein 1 in the paclitaxel-resistant phenotype. *Clin Cancer Res.* 1999;**5**(11):3445-3453.
19. Tellez-Gabriel M, Ory B, Lamoureux F, Heymann M-F, Heymann D. Tumour heterogeneity: the key advantages of single-cell analysis. *Int J Mol*. 2016;**17**(12):2142. doi: 10.3390/ijms17122142
20. Pils D, Horak P, Gleiss A, Sax C, Fabjani G, Moebus VJ, *et al.*

Five genes from chromosomal band 8p22 are significantly down regulated in ovarian carcinoma. *Cancer.* 2005;**104**(11):2417-2429. doi: 10.1002/cncr.21538

21. Kanamarlapudi V. Exchange factor EFA6R requires C-terminal targeting to the plasma membrane to promote cytoskeletal rearrangement through the activation of ADP-ribosylation factor 6 (ARF6). *J Biol Chem*. 2014;**289**(48):33378-33390. doi: 10.1074/jbc.M113.534156
22. Westphalen CB, Quante M, Wang TC. Functional implication of Dclk1 and Dclk1-expressing cells in cancer. *Small GTPases.* 2017;**8**(3):164-671. doi: 10.1080/21541248.2016.1208792
23. Chandrakesan P, Panneerselvam J, Qu D, Weygant N, May R, Bronze M, *et al*. Regulatory roles of Dclk1 in epithelial mesenchymal transition and cancer stem cells. *J Carcinog Mutagen.* 2016;**7**(2). doi: 10.4172/2157-2518.1000257
24. Sureban SM, May R, Weygant N, Qu D, Chandrakesan P, Bannerman-Menson E, *et al*. XMD8-92 inhibits pancreatic tumor xenograft growth via a DCLK1-dependent mechanism. Cancer letters. 2014;**351**(1):151-161. doi: 10.1016/j.canlet.2014.05.011
25. Sureban SM, May R, Mondalek FG, Qu D, Ponnurangam S, Pantazis P, *et al.* Nanoparticle-based delivery of siDCAMKL-1 increases microRNA-144 and inhibits colorectal cancer tumor growth via a Notch-1 dependent mechanism. *J Nanobiotechnology.* 2011;**9**(1):40. doi: 10.1186/1477-3155-9-40
26. Korpal M, Lee ES, Hu G, Kang Y. The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. *J Biolog Chem.* 2008;**283**(22):14910-14914.
27. Roybal JD, Zang Y, Ahn Y-H, Yang Y, Gibbons DL, Baird BN, *et al.* miR-200 Inhibits lung adenocarcinoma cell invasion and metastasis by targeting Flt1/VEGFR1. *Mol Cancer Res.* 2011;**9**(1):25-35. doi: 10.1158/1541-7786.MCR-10-0497
28. Puiffe M-L, Le Page C, Filali-Mouhim A, Zietarska M, Ouellet V, Tonin PN, *et al.* Characterization of Ovarian Cancer Ascites on Cell Invasion, Proliferation, Spheroid Formation, Gene Expression in an *In Vitro* Model of Epithelial Ovarian Cancer. *Neoplasia.* 2007;**9**(10):820-IN828. doi: 10.1593/neo.07472
29. Hsu F-H, Serpedin E, Hsiao T-H, Bishop AJ, Dougherty ER, Chen Y, editors. Identifying genes associated with chemotherapy response in ovarian carcinomas based on DNA copy number and expression profiles. GENSIPS , 2011 IEEE International Workshop on; 2011: *IEEE*. doi: 10.1109/GENSiPS.2011.6169438
30. Wang C, Xiao Y, Hu Z, Chen Y, Liu N, Hu G. PEG10 directly regulated by E2Fs might have a role in the development of hepatocellular carcinoma. *FEBS letters.* 2008;**582**(18):2793-2798. doi: 10.1016/j.febslet.2008.07.009
31. ZITAN Q, XUz Z. CHML suppresses cell growth and induces apoptosis in multiple human tumor lines. *Anticancer Res.* 1999;**19:**2893-2900.
32. Barøy T, Chilamakuri CS, Lorenz S, Sun J, Bruland ØS, Myklebost O, *et al.* Genome Analysis of Osteosarcoma Progression Samples Identifies FGFR1 Overexpression as a Potential Treatment Target and CHM as a Candidate Tumor Suppressor Gene. *PloS One.* 2016;**11**(9):e0163859. doi: 10.1371/journal.pone.0163859
33. Neilsen PM, Noll JE, Mattiske S, Bracken CP, Gregory PA, Schulz RB, *et al.* Mutant p53 drives invasion in breast tumors through up-regulation of miR-155. *Oncogene.* 2013;**32**(24):2992. doi: 10.1038/onc.2012.305
34. Dankert JF, Pagan JK, Starostina NG, Kipreos ET, Pagano M. FEM1 proteins are ancient regulators of SLBP degradation. *Cell Cycle.* 2017;**16**(6):556-564. doi: 10.1080/15384101.2017.1284715
35. Coyle-Thompson CA, Banerjee U. The strawberry notch gene functions with Notch in common developmental pathways. *Development.* 1993;*119*(2):377-395. doi: 10.1242/dev.119.2.377
36 Suzuki C, Takahashi K, Hayama S, Ishikawa N, Kato T, Ito T, *et al.* Identification of Myc-associated protein with JmjC domain as a novel therapeutic target oncogene for lung cancer. *Mol Cancer Ther.* 2007;**6**(2):542-551. doi: 10.1158/1535-7163.MCT-06-0659
37. Kavela S, Shinde SR, Ratheesh R, Viswakalyan K, Bashyam MD, Gowrishankar S, *et al*. PNUTS functions as a proto-oncogene by sequestering PTEN. *CancerRes.* 2013;**73**(1):205-214.. doi: 10.1158/0008-5472.CAN-12-1394
38. Mastropasqua F, Marzano F, Valletti A, Aiello I, Di Tullio G, Morgano A, *et al.* TRIM8 restores p53 tumour suppressor function by blunting N-MYC activity in chemo-resistant tumours. *Mol Cancer.* 2017;**16**(1):67. doi: 10.1186/s12943-017-0634-7
39. Arango D, Corner GA, Wadler S, Catalano PJ, Augenlicht LH. c-myc/p53 interaction determines sensitivity of human colon carcinoma cells to 5-fluorouracil *in vitro* and *in vivo*. *Cancer Res.* 2001;**61**(12):4910-4915.
40. VanKlompenberg MK, Leyden E, Arnason AH, Zhang J-T, Stefanski CD, Prosperi JR. APC loss in breast cancer leads to doxorubicin resistance via STAT3 activation. *Oncotarget.* 2017;**8**(61):102868. doi: 10.18632/oncotarget.22263
41. Castellano G, Torrisi E, Ligresti G, Malaponte G, Militello L, Russo AE, *et al.* The involvement of the transcription factor Yin Yang 1 in cancer development and progression. *Cell Cycle.* 2009;**8**(9):1367-1372. doi: 10.4161/cc.8.9.8314
42. Kashyap V, Bonavida B. Role of YY1 in the pathogenesis of prostate cancer and correlation with bioinformatic data sets of gene expression. *Genes Cancer.* 2014;**5**(3-4):71. doi: 10.18632/genesandcancer.12
43. Godwin P, Baird A-M, Heavey S, Barr M, O'Byrne K, Gately KA. Targeting nuclear factor-kappa B to overcome resistance to chemotherapy. *Frontiers in oncology.* 2013;**3**:120. doi: 10.3389/fphar.2012.00202
44. Lam E-F, Francis R, Petkovic M. FOXO transcription factors: key regulators of cell fate. *Portland Press Limited;* 2006. doi: 10.1042/BST0340722
45. Gomes AR, Brosens JJ, Lam EW-F. Resist or die: FOXO transcription factors determine the cellular response to chemotherapy. *Cell Cycle.* 2008;**7**(20):3133-3136. doi: 10.4161/cc.7.20.6920
46. Zheng H, Bae Y, Kasimir-Bauer S, Tang R, Chen J, Ren G, *et al.* Therapeutic antibody targeting tumor-and osteoblastic

niche-derived jagged1 sensitizes bone metastasis to chemotherapy. *Cancer Cell.* 2017;**32**(6):731-747. doi: 10.1016/j.ccell.2017.11.002

47. Li C-M, Margolin AA, Salas M, Memeo L, Mansukhani M, Hibshoosh H, *et al*. PEG10 is a c-MYC target gene in cancer cells. *Cancer Res*. 2006;**66**(2):665-672. doi: 10.1158/0008-5472.CAN-05-1553
48. Barry SP, Townsend PA, Knight RA, Scarabelli TM, Latchman DS, Stephanou A. STAT3 modulates the DNA damage response pathway. *Int J Exp Pathol.* 2010;**91**(6):506-514. doi: 10.1111/j.1365-2613.2010.00734.x
49. Coster G, Goldberg M. The cellular response to DNA damage: a focus on MDC1 and its interacting proteins. *Nucleus*. 2010;**1**(2):166-178. doi: 10.4161/nucl.1.2.11176
50. Ambros V. The functions of animal microRNAs. *Nature.* 2004;**431**(7006):350. doi: 10.1038/nature02871
51. Hu YW, Kang CM, Zhao JJ, Nie Y, Zheng L, Li HX, *et al.* Lnc RNA PLAC 2 down regulates RPL 36 expression and blocks cell cycle progression in glioma through a mechanism involving STAT 1. *J Cell Mol Med*. 2018;**22**(1):497-510. doi: 10.1111/jcmm.13338
52. Jiang Z, Dong X, Li Z-G, He F, Zhang Z. Differential coexpression analysis reveals extensive rewiring of Arabidopsis gene coexpression in response to Pseudomonas syringae infection. *Sci Rep.* 2016;**6**:35064. doi: 10.1038/srep35064
53. Stavropoulou V, Xie J, Henriksson M, Tomkinson B, Imreh S, Masucci MG. Mitotic infidelity and centrosome duplication errors in cells overexpressing tripeptidyl-peptidase II. *Cancer Res*. 2005;**65**(4):1361-1368. doi: 10.1158/0008-5472.CAN-04-2085
54. Duensing S, Darr S, Cuevas R, Melquiot N, Brickner AG, Duensing A, *et al*. Tripeptidyl Peptidase II Is Required for c-MYC–Induced Centriole Overduplication and a Novel Therapeutic Target in c-MYC–Associated Neoplasms. *Genes Cancer.* 2010;**1**(9):883-892. doi: 10.1177/1947601910389605