

## ORIGINAL ARTICLE

# Identification of dysregulated miRNAs-genes network in ovarian cancer: An integrative approach to uncover the molecular interactions and oncomechanisms

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**Abstract**

**Background:** Ovarian (OV) cancer is considered as one of the most deadly malignancies in women, since it is unfortunately diagnosed in advanced stages. Nowadays, the importance of bioinformatics tools and their frequent usage in tracking dysregulated cancer-related genes and pathways have been highlighted in researches.

**Aim:** The aim of this study is to investigate dysregulated miRNAs-genes network and its function in OV tumors based on the integration of microarray data through a system biology approach.

**Methods:** Two microarray data (GSE119056 and GSE4122) were analyzed to explore the differentially expressed miRNAs (DEmiRs) and genes among OV tumors and normal tissues. Then, through the help of TargetScan, miRmap, and miRTarBase databases, the dysregulated miRNA-gene network in OV tumors was constructed by Cytoscape. In the next step, co-expression and protein-protein interaction networks were made using GEPIA and STRING databases. Moreover, the functional analysis of the hub genes was done by DAVID, KEGG, and Enrichr databases. Eventually, the regulatory network of TF-miRNA-gene was constructed.

**Results:** The potential dysregulated miRNAs-genes network in OV tumors has been constructed, including 109 differentially expressed genes (DEGs), 25 DEmiRs, and 213 interactions. Two down-regulated microRNAs, miR-660-3p and hsa-miR-4510, have the most interactions with up-expressed oncogenic DEGs. CDK1, PLK1, CCNB1, CCNA2, and EZH2 are involved in protein module, which show significant overexpression in OV tumors according to The Cancer Genome Atlas (TCGA) data. EZH2 shows amplification in OV tumors with remarkable percentage. The transcription factors TFAP2C and GATA4 have the pivotal regulatory functions in oncotranscriptomic profile of OV tumors.

**Conclusion:** In current study, we have collected and integrated different data to uncover the complex molecular interactions and oncomechanisms in OV tumors. The DEmiRs-DEGs and TF-miRNA-gene networks reveal the potential interactions that could be a significant piece of the OV onco-puzzle.

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## KEYWORDS

bioinformatics, microarray data, miRNA, ovarian cancer, systems biology

## 1 | INTRODUCTION

Ovarian (OV) cancer shows the seventh most frequency among the malignancies in women.<sup>1</sup> In 2018, around the world, 295 414 new cases of this disease and 184 799 OV cancer-related death approximately registered.<sup>2</sup> Unfortunately, this cancer is diagnosed very late, and this factor strongly decreases the lifetime of patients. However, early diagnosis and effective treatment strategies could notably improve the patient's lifetime.<sup>3</sup>

Increasing evidence indicates that miRNAs, as a category of short noncoding RNAs, alongside coding RNAs play pivotal roles in cancer initiation and progression. It is well established that microRNAs are involved in complex posttranscriptional gene regulatory network in both roles of tumor suppressor or oncogenic miRNAs (oncomiRs).<sup>4</sup>

The incredible improvements in high-throughput technologies such as microarray and RNA-seq as well as bioinformatics tools generate huge data concerning coding and noncoding transcripts in the field of cancer research. Although, interpretation and intersection of available data are main challenges in this context.<sup>5</sup> Despite the extensive studies about molecular mechanisms in pathogenesis of OV cancer, the molecular interactions of coding and noncoding transcripts in the complex gene regulatory network have not completely figured out.

The purpose of this study is the combination of microarray data and bioinformatics analyses for a better understanding of dysregulated key genes and miRNAs in OV cancer compared with normal tissues. In this study, we constructed dysregulated miRNAs-genes, co-expression, and protein-protein interactions (PPIs) networks. The identified hub genes and their interactions could clarify underlying factors in OV cancer development and present efficient therapeutic targets.

## 2 | METHODS

### 2.1 | Differentially expressed genes and miRNAs (DEmiRs) identification

Two Gene Expression Omnibus (GEO) datasets including GSE119056 (GPL19615 platform) with six tumor and three normal samples as well as GSE4122 with 32 tumor and 14 normal specimens were selected and analyzed. The Benjamini and Hochberg false discovery rate (FDR) method was used to adjust the *P*-value to obtain the multiple testing adjusted *q*-value. All the data are normalized and cross-comparable (Figure S1). For selecting differentially expressed genes (DEGs) in OV tumors compared with normal tissues, adj. *P*-value < .05 and log FC  $\geq |2|$  were set. Then the common genes between both GSEs that show the same expression direction (up- or down-expression) were chosen for the next step.

GSE119056 (GPL21572 platform) for investigation of DEmiRs between OV cancer and nontumor OV tissues was analyzed. Similar to the prior step, adj. *P*-value < .05 and log FC  $\geq |2|$  criteria were considered.

### 2.2 | DEG-DEmiR interactions

In the next step, the bioinformatically predicted and experimentally validated DEG-DEmiR interactions were obtained using TargetScan,<sup>6</sup> miRmap,<sup>7</sup> and miRTarBase<sup>8</sup> databases. It should be noted that the predicted interactions that exist in both TargetScan and miRmap were selected. Finally, the dysregulated miRNAs-genes network was constructed including experimentally validated DEG-DEmiR interactions based on miRTarBase database and predicted interactions by Cytoscape v3.6.1.<sup>9</sup>

### 2.3 | Co-expression and protein-protein interaction networks

The co-expression network was visualized based on the top 20 genes that are co-expressed with each of DEGs according to TCGA data; GEPIA web server was used to retrieve the co-expressed genes of DEGs.<sup>10</sup> The PPI network including DEGs and their co-expressed genes was constructed using String database.<sup>11</sup> The genes with the higher betweenness centrality and degree were determined as the most important genes in these networks (hub genes). Eventually, the functions of the hub genes were identified by DAVID<sup>12</sup> and Enrichr<sup>13</sup> databases.

To detect the main protein module in PPI network, MCODE app,<sup>14</sup> the Cytoscape plugin, was run and the pathway in which this protein complex is involved was detected by KEGG mapper.<sup>15</sup>

### 2.4 | Oncogenomic and oncotranscriptomic analysis

The genetic alterations of the PPI network hub genes in the 606 OV tumors were retrieved from cBioPortal database (TCGA provisional).<sup>16</sup> To compare expression of hub genes between OV tumors and adjacent normal tissues based on TCGA data as well as ovarian normal tissues based on the Genotype-Tissue Expression (GTEx) data, GEPIA web server was used. The method for differential analysis is one-way ANOVA; genes with  $|\log_2FC| > 1$  and *q*-value < 0.01 are considered DEGs.

### 2.5 | Drug-protein network

To investigate the potential of hub genes to becoming therapeutic targets, the drugs-protein interactions were detected by Cytoscape linker.<sup>17</sup>

## 2.6 | Identification of TF-miRNA-gene axes

We used HOCOMOCO v11 database (comprehensive collection of human transcription factor [TF]) to identify our DE genes that act as TF.<sup>18</sup> Then TransmiR v2.0 (an experimentally supported TF-miRNA regulatory relationship database) was used to identify TF-miRNA interactions.<sup>19</sup> TRRUST v2 (transcriptional regulatory relationships unraveled by sentence-based text mining)<sup>20</sup> and RegNetwork (an integrated database of transcriptional and posttranscriptional regulatory networks in human and mouse)<sup>21</sup> databases were applied to assess the TF-TF and TF-gene interactions.

119056 and GSE4122. The number of common DEGs with adj.  $P$ -value  $< .05$  and  $\log FC \geq |2|$  is 109 which shows same direction of expression in two datasets (Table S1). Also, after analyzing of GSE119056, 25 DEmiRs with adj.  $P$ -value  $< .05$  and  $\log FC \geq |2|$  in OV cancer tissues were screened (Table S2).

To obtain dysregulated miRNA-mRNA axes in OV tumors, three databases (TargetScan and miRmap for predicted interactions and miRTarBase for experimentally validated interactions) were applied. A total of 176 common predicted interactions in two mentioned databases and 46 validated interactions were identified. Taken together, 213 interactions have been detected (Table S3). Finally, DEG-DEmiR network in OV tumors has been visualized by Cytoscape (Figure 1).

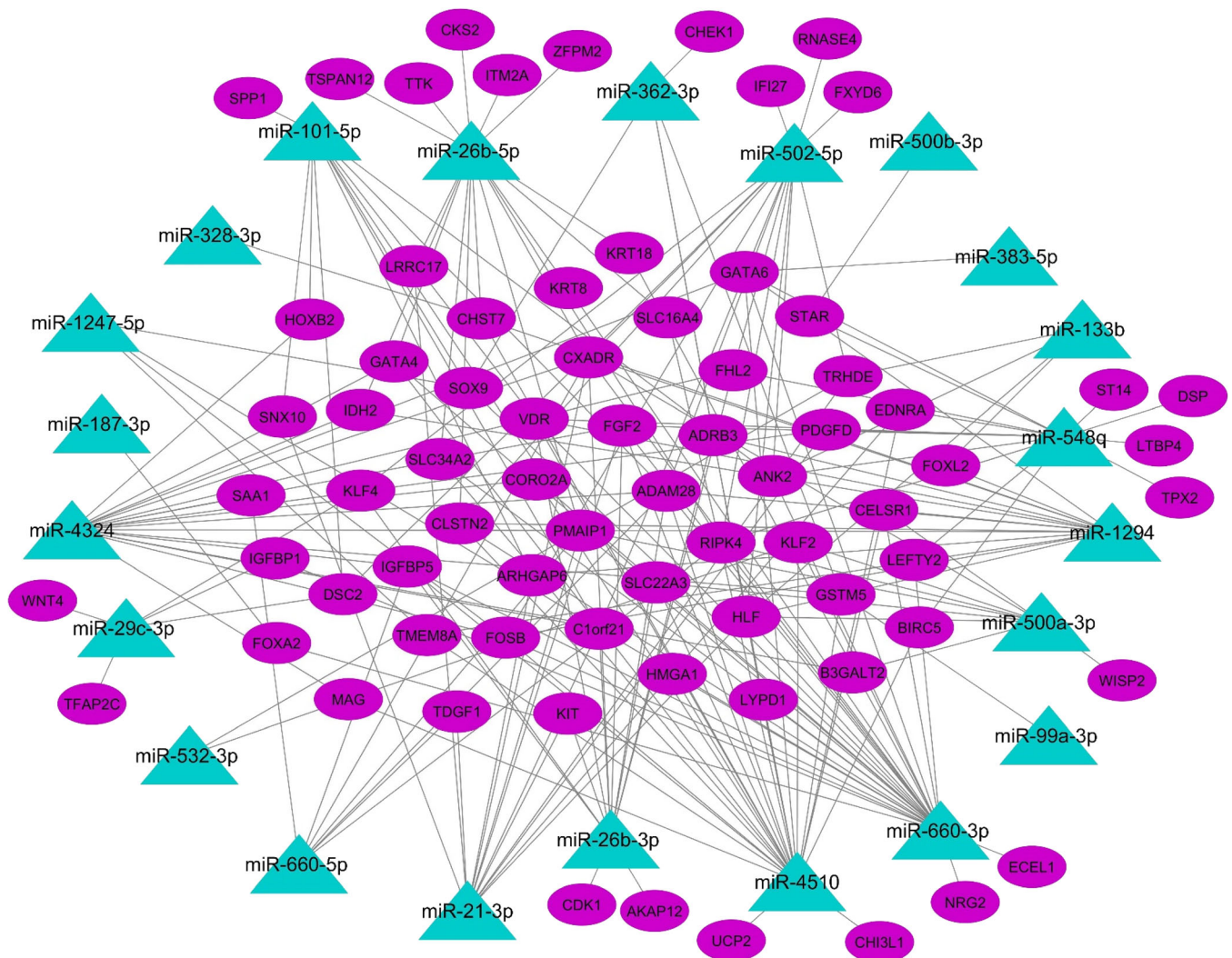
## 3 | RESULTS

### 3.1 | DEG-DEmiR network

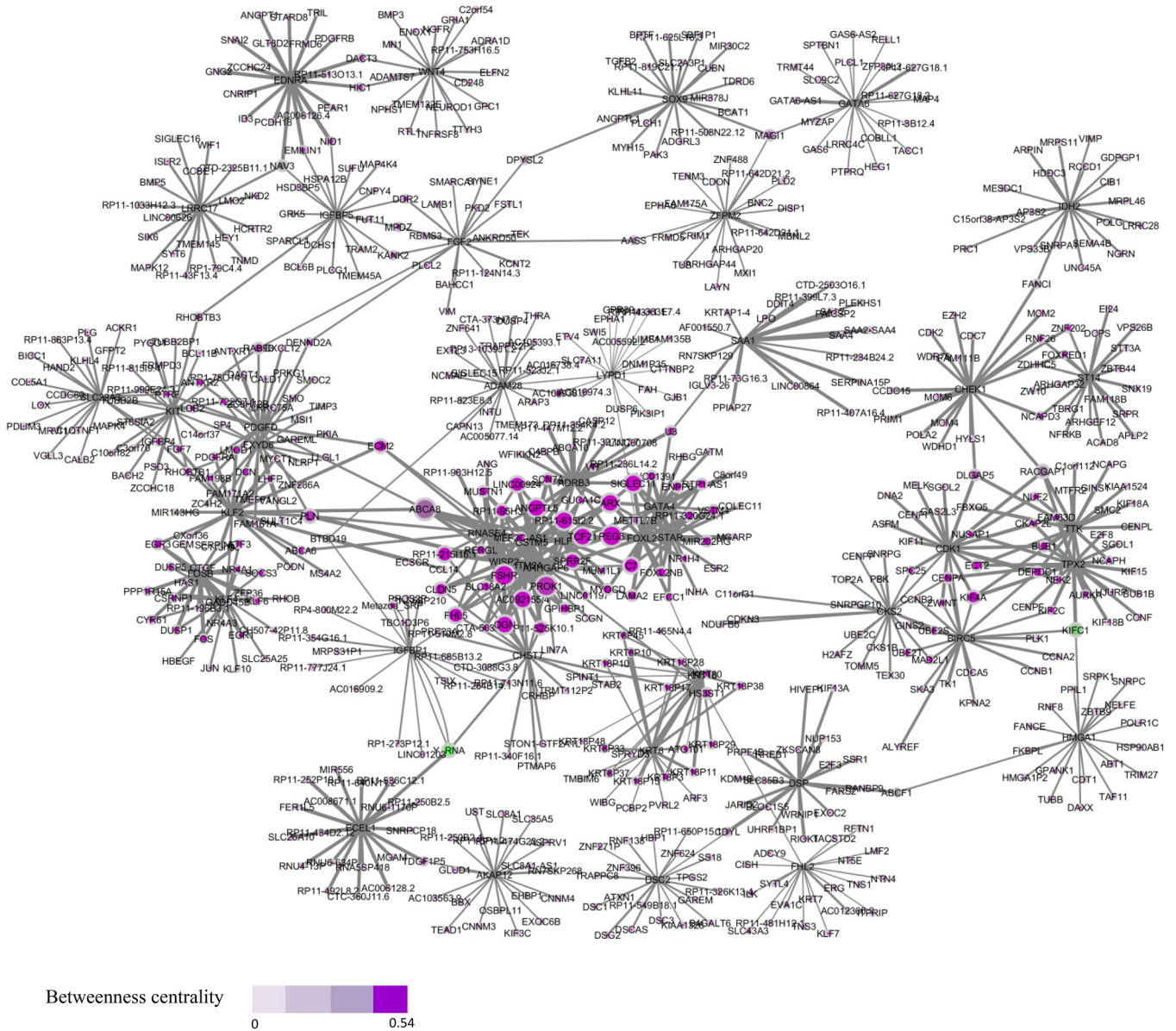
As it was mentioned before, DEGs in OV cancer samples compared with normal tissues were identified through analyzing two GSE

### 3.2 | Co-expression network

The 20 top co-expressed genes of each DEGs were retrieved from GEPIA web server. Co-expression network consists of DEGs along with their co-expressed genes. ABCA8, ECM2, KIFC1, MAGI1, and



**FIGURE 1** Dysregulated miRNA-gene network. The cyan triangles and purple ellipses present miRNAs and genes, respectively. The potential molecular interactions between miRNAs and mRNAs are shown by lines



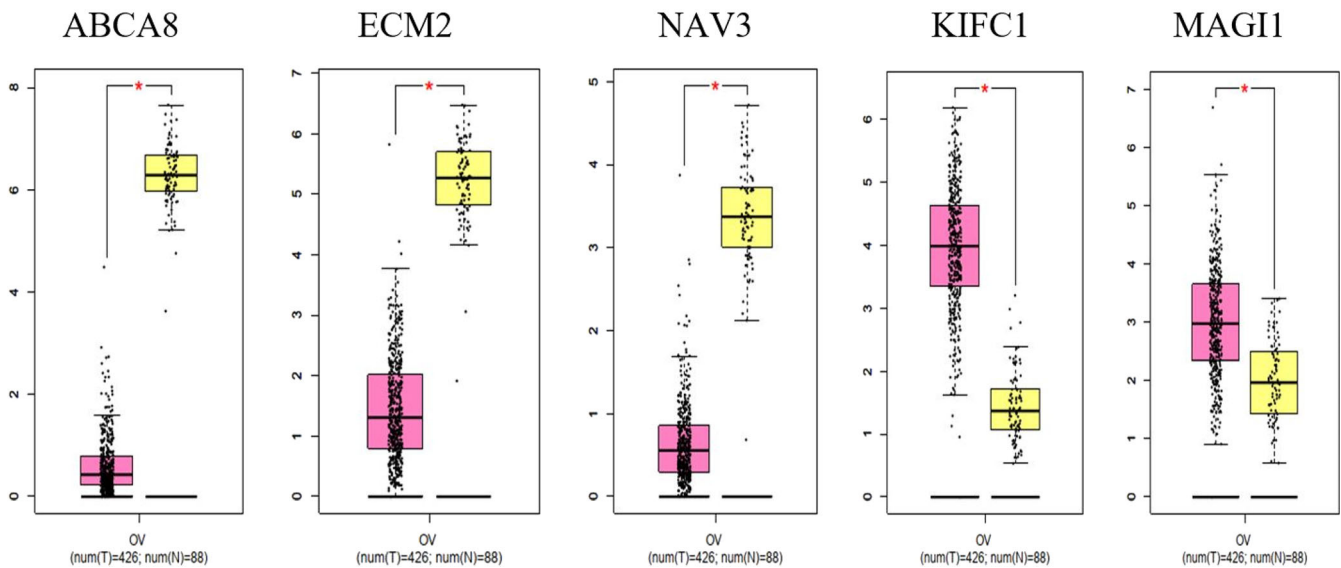
**FIGURE 2** The co-expression network including DEGs and their top 20 co-expressed genes in OV cancer. The color and size of nodes indicate betweenness centrality and degree, respectively. Degree has a range from 1 to 26 for GSTM5 gene. The width of the edges has been adjusted according to PCC. The nodes with a higher size and stronger color are considered as hub genes. DEG, differently expressed gene; OV, ovarian; PCC, Pearson correlation coefficient

NAV3 genes with the highest degree and betweenness centrality are selected as hub genes (Figure 2). The expression of these hubs in OV cancer based on TCGA and GTEx data shows that all of them are significantly dysregulated in tumors compared with normal tissues (Figure 3).

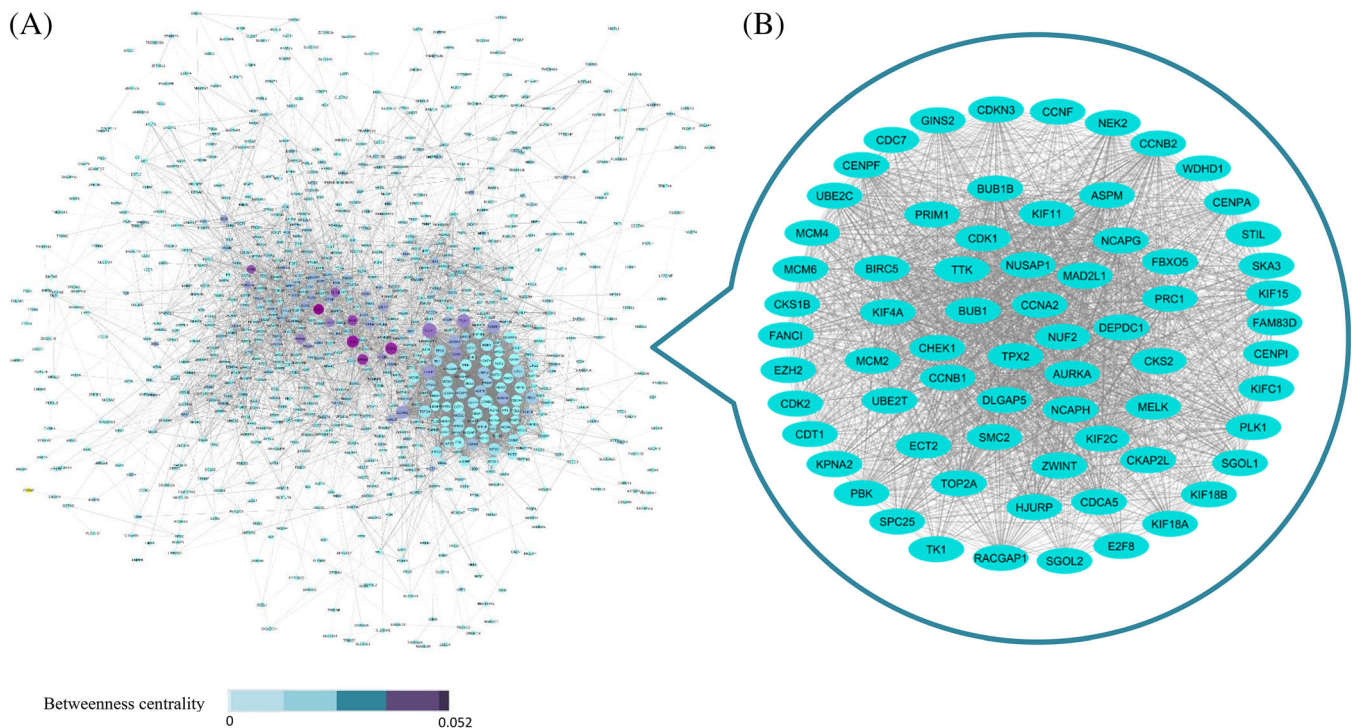
### 3.3 | Protein-protein interaction network

In the next step, for preparing of PPI network, the STRING database was run. CDK1, PLK1, CCN1, CCNA2, EZH2, and AURKA show the

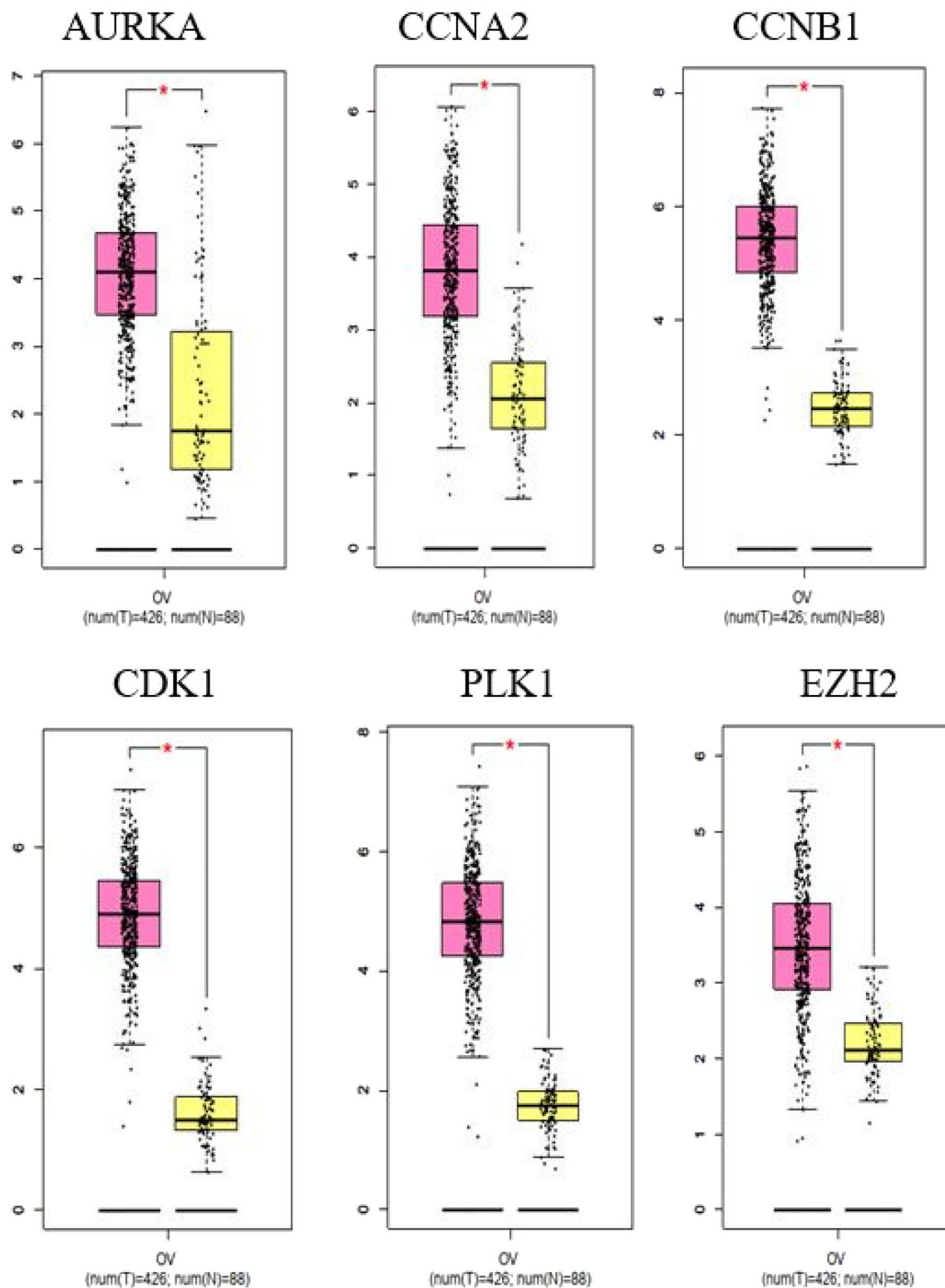
ability to be hub genes because of having a higher degree and betweenness centrality (Figure 4A). Then the box plots related to expression difference of these six hub genes between OV tumors and normal samples were analyzed by GEPIA database based on TCGA and GTEx data (Figure 5). All the hub genes show the significant expression differences between two sample groups. The genetic alterations of hub genes in 606 OV cancer samples were investigated by cBioPortal database. EZH2 and AURKA have a considerable mutation rate (9% and 11% of ovarian tumors, respectively), which most of them are gene amplification. Functional annotation according to Enrichr revealed the role of hub genes in cyclin-dependent protein



**FIGURE 3** The boxplots of the expression changes of co-expression network hub genes (ABCA8, ECM2, NAV3, CDK1, KIFC1, and MAGI1) in the OV tumor tissues compared with normal samples based on TCGA and GTEx data. The pink and yellow boxes show the tumor and nontumor tissues, respectively. These data have been obtained from GEPIA web server. Genes with  $|\log_2FC| > 1$  and  $q$ -values  $< 0.01$  are considered differentially expressed genes. The red star shows a significant difference between the tumors and nontumor tissues. GTEx, Genotype-Tissue Expression; OV, ovarian; TCGA, The Cancer Genome Atlas

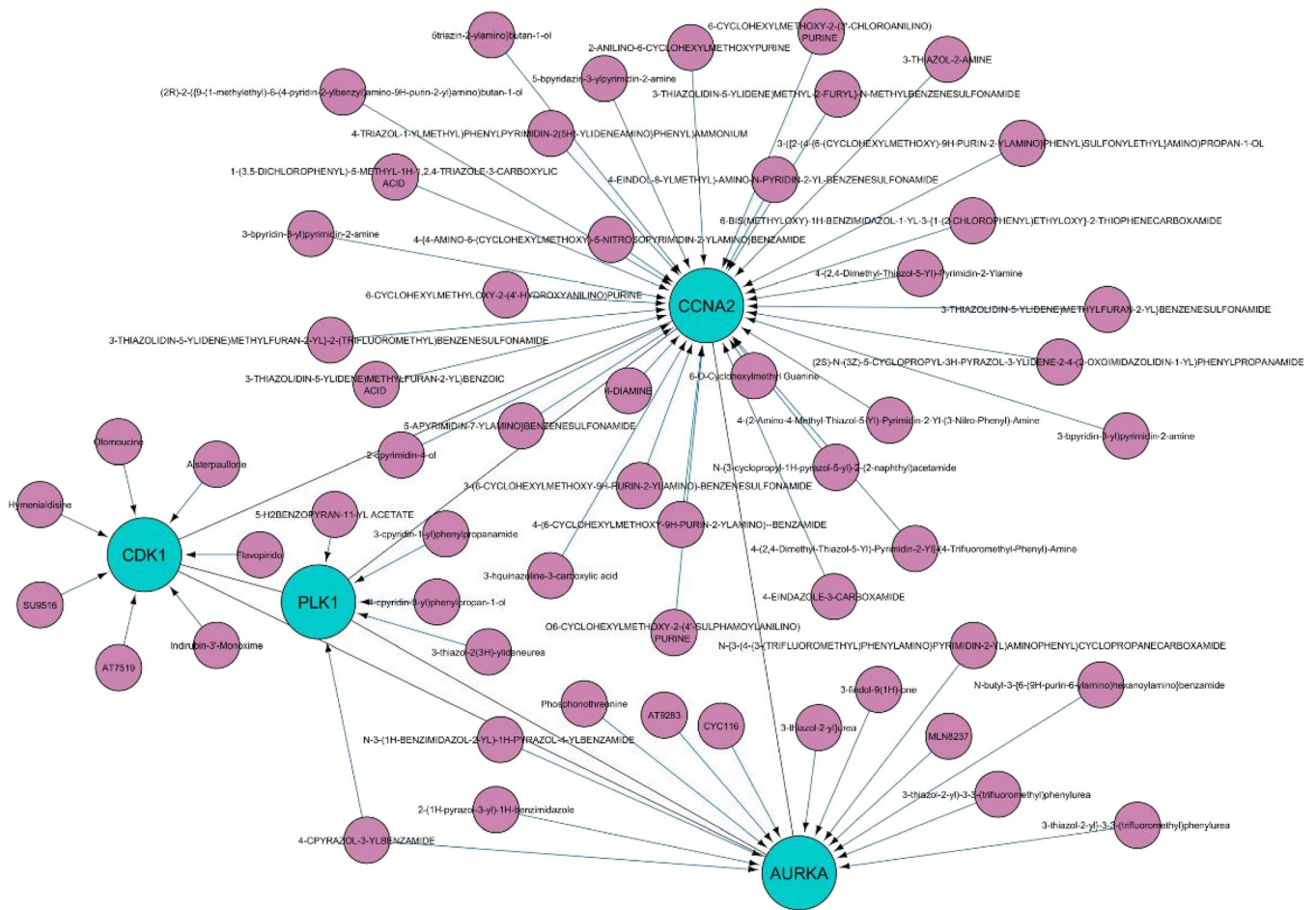


**FIGURE 4** A, Protein-protein interaction network based on DEGs and their co-expressed genes in OV tumors. The color and size of nodes refer to betweenness centrality and degree, respectively. Degree has a range from 1 to 114 for CDK1 gene. The width of edges was adjusted based on the combined scores. B, The main protein module of PPI according to MCODE app. DEG, differentially expressed gene; OV, ovarian; PPI, protein-protein interaction



**FIGURE 5** The boxplots of expression of PPI network hub genes (AURKA, CCNA2, CCNB1, CDK1, PLK1, and EZH2) in OV tumors compared with normal samples. The pink and yellow boxes represent the tumor and normal tissues, respectively. These data obtained from GEPIA database based on TCGA and GTEx data. Genes with  $|\log_2FC| > 1$  and  $q$ -values  $< 0.01$  are considered differentially expressed genes. The red star shows a significant difference between tumors and matched nontumor tissues ( $P$ -value  $\leq .05$ ). GTEx, Genotype-Tissue Expression; OV, ovarian; PPI, protein-protein interaction; TCGA, The Cancer Genome Atlas





**FIGURE 8** The drug-protein network. The gray and purple ellipses show the genes and drugs, respectively. Data have been visualized by Cytoscape linker app

### 3.5 | Dysregulated TF-miRNA-gene network

Among 74 DE genes in miRNA-gene network, 11 genes (VDR, HOXB2, GATA4, FOSB, GATA6, KLF4, HLF, FOXA2, SOX9, HMGA1, and TFAP2C) were identified as TFs by HOCOMOCO database. Twenty-one validated interactions between TFs and miRNAs have been uncovered by running TransmiR database (Table S6). Also, 21 interactions between TF-TF and TF-gene have been detected by TRUST and RegNetwork databases (Table S6). Eventually, dysregulated TF-miRNA-gene network in OV tumors has been visualized by Cytoscape (Figure 9).

## 4 | DISCUSSION

Among the cancer-related deaths in women, OV cancer is ranked fifth. The contribution of genetic factors and family history to this cancer is well known.<sup>22</sup> Nowadays, the importance of noncoding RNAs in various malignancies is becoming more and more defined. The role of miRNAs as a tumor suppressors or oncogenes has been ascertained in tumor initiation and progression.<sup>23</sup> Despite their short length (up to

24 nucleotides), they are involved in the regulation of many genes expression.<sup>24</sup> In this study, through analyzing two microarray data (GSE119056 and GSE4122), 109 common DEGs in OV tumors compared with normal tissues were obtained with adj. *P*-value < .05 and log FC ≥ |2|. Moreover, according to GSE119056, 25 DEmiRs with adj. *P*-value < .05 and log FC ≥ |2| were detected in OV cancer. The DEGs-DEmiRs network in OV tumors has been constructed through an integrative approach. This network consists of 213 mRNA-miRNA axes.

The DEGs-DEmiRs network uncovers the potential tumor suppressor miRNAs that have not reported in OV cancer so far. Two down-regulated miRNAs named miR-660-3p and hsa-miR-4510 show the most interactions with DEGs. Both of them in our study could target some studied oncogenes in other studies including ADAM28,<sup>25</sup> HMGA1<sup>26</sup> and SLC34A2.<sup>27</sup>

According to Shyian et al report, the relationship between expression of SLC34A2 and differentiation level of OV tumor cells was confirmed. So, it can be used as a possible marker in determination of cell differentiation.<sup>28</sup> Also, the direct link of HMGA1 expression with OV tumor grade and proliferation rate in related cell lines was shown by several techniques.<sup>29</sup> Furthermore, miR-660-3p is probably involved





**TABLE 1** Summary of dysregulated miRNAs in different studies related to OV cancer

Transcript	Study	Dysregulation	Methods
miRNA-328-3p	Wang et al <sup>36</sup>	Up	qRT-PCR
	Srivastava et al <sup>37</sup>	Up	qPCR, microarray analysis, luciferase reporter assay, immunoblotting, ALDH analyses, sphere-forming assay, xenograft tumor study
miRNA-21-3p	Báez-Vega et al <sup>38</sup>	Up	qPCR, colony formation assays, in vitro invasion assay, cell viability assay, western blot analysis, luciferase assays, immunohistochemistry
miRNA-133p	Liu et al <sup>39</sup>	Down	RT-PCR, cell viability assay, cell proliferation assay, transwell invasion assay, western blot analysis, luciferase reporter assay
	Yang et al <sup>40</sup>	Down	RT-PCR, western blot assay, invasive and migration assays
miRNA-1294	Guo et al <sup>41</sup>	Down	qRT-PCR, cell counting kit-8 assay, cell cycle analysis
	Zhang et al <sup>42</sup>	Down	qRT-PCR, MTT assays, wound healing, tumor invasion assays, western blot
miRNA-383-5p	Jiang et al <sup>43</sup>	Down	qRT-PCR, western blot assay, IHC, dual-luciferase reporter assay, cell proliferation assay, Edu incorporation assay, animal models
miRNA-532-3p	Huang et al <sup>44</sup>	Down	RT-qPCR, cell proliferation assay, colony formation assay, Edu incorporation assay, transwell assay, scratch wound assay, luciferase reporter gene assay, RIP assay
miRNA-500b-3p	Pandey et al <sup>45</sup>	Down	qRT-PCR

Abbreviations: ALDH, aldehyde dehydrogenase; IHC, immunohistochemistry; OV, ovarian; qPCR, quantitative PCR; RIP, RNA immunoprecipitation; RT-PCR, real-time polymerase chain reaction.

ABCA8 is down-regulated in several drug-resistant OV cancer cell lines.<sup>51</sup>

Kinesin family member C1 (KIFC1) plays an indispensable performance in mitotic spindle assembly, mitotic sister chromatid segregation, and cell division (Table S4). According to previous reports, KIFC1 in people with OV cancer is overexpressed and significantly related to advanced stage and grade of tumors and patient's poor survival.<sup>52,53</sup> Also, the oncogenic role of KIFC1 in breast cancer was clarified. The suppression of this gene by poly ADP-ribose polymerase in breast cancer cell lines and decreasing of their livability is the evidence of this claim.<sup>54</sup> These findings and our analyses indicate the remarkable roles of KIFC1 in oncotranscriptomic profile of ovarian cancer and its value to becoming therapeutic target. Interestingly, Zhang et al introduced an inhibitor of KIFC1 for the interdicting of its role in centrosome clustering in malignant cells that this finding accelerates the targeted cancer cell death without normal cell affection.<sup>55</sup>

According to DAVID, the extracellular matrix protein 2 (ECM2) participates in positive regulation of cell-matrix adhesion, axonogenesis, and extracellular matrix organization (Table S4). The high expression of ECM2 in normal OV epithelium and its down-expression in OV tumors have been identified through gene expression profiling.<sup>56</sup> Result of our analysis according to TCGA and GTEx data is consistent with previous reports.

Another hub gene in the co-expression network is neuron navigator 3 (NAV3). Its suppression in the OV cancer cell lines by miRNA-

21-3p results in cisplatin resistance.<sup>57</sup> Moreover, in another study, NAV3 was identified as a target of miRNA-429. In OV cell lines, which are transfected by miRNA-429, NAV3 showed down-expression and the metastatic ability of tumors reversed.<sup>58</sup> Our results show that this gene is significantly down-regulated in OV cancers. Furthermore, in colon and squamous cell cancers, NAV3 copy number variations (deletion and amplification) are a common phenomenon and affects cell invasion.<sup>59,60</sup>

Membrane-associated guanylate kinase (MAGI1) plays important roles in protein complex assembly, cell adhesion, and cell surface receptor signaling pathway (Table S4). This gene shows the significant overexpression in OV tumors according to TCGA data. However, Kori et al identified MAGI1 as one of down-expressed DEGs in their microarray findings in OV cancer samples.<sup>61</sup>

Taken together, in oncotranscriptomic profile of OV tumors, two down-expressed hub genes including ABCA8 and NAV3 have the key role in drug-resistant tumors and should be monitored as long as cancer therapy is concerned.

In current study, the PPI network has been constructed which consists of DEGs in two GEO datasets and their co-expressed genes. In this network, CDK1, PLK1, CCNB1, CCNA2, EZH2, and AURKA are considered as hub genes. All of these genes have a higher expression in OV tumors relative to normal tissues according to TCGA and GTEx data. The common targets that are present in both DEG PPI and miRNA networks were collected in Table S5. In 2017, Zhang et al

showed CDK1 inhibition results in repression of cell proliferation of OV cancer cell lines.<sup>62</sup> Moreover, it was demonstrated that CDK1 is a target of miRNA-490-3p. This miRNA plays a tumor suppressive role in OV cancer cell line through inhibiting CDK1.<sup>63</sup> Another hub gene in the PPI network is PLK1 protein. When PLK1 is suppressed, OV cell propagation and proliferation are decreased.<sup>64</sup> In addition, the roles of PLK1 in the elevation of autophagy and drug resistance were elucidated in ovarian clear cell carcinoma.<sup>65</sup> According to Jiang et al study, the attenuation of FOXM1 and PLK1 results in reinforcing P21 amount and finally OV cell apoptosis.<sup>66</sup> PLK1 and CCNB1 have been introduced as downstream targets of FOXM1 TF, and all of them are overexpressed in OV tumors.<sup>67</sup> Both of the CCNB1 and CCNA2 are members of cyclin family genes and promote transition through G1/S and G2/M in the cell cycle. CCNA2 shows high expression in OV tumors and association with insensitivity to chemotherapy.<sup>68</sup> AURKA gene product is a protein kinase that shows to be involved in microtubule formation during chromosome segregation. The role of AURKA gene in the migration of OV cancer cells has been uncovered.<sup>69</sup> The EZH2 gene codes a histone methyltransferase which participates in histone methylation and consequently in transcriptional activity. Gain-of-function mutations and overexpression of EZH2 have been linked to many forms of cancer.<sup>70,71</sup>

It is shown that one reason of resistance to cisplatin in OV cancer patients is activation of the cMyc/miRNA-137/EZH2 axis. In normal cells, miR-137 suppresses EZH2, but cMyc by trying to neutralize this inhibition reinforces the ability of resistance in malignant cells.<sup>72</sup> Interestingly, our investigation concerning EZH2 genetic alterations in OV tumors reveals an amplification in remarkable percentage of patients (11%). These data could justify EZH2 overexpression in a portion of OV cancer patients. Moreover, the detected protein module contains CDK1, PLK1, CCNB1, CCNA2, and EZH2. We show that this module is involved in the progression of different phases of the cell cycle. All the evidence considered, we demonstrated that CDK1, PLK1, CCNB1, CCNA2, AURKA, and EZH2 not only are hub nodes in PPI network but also are significantly overexpressed in OV tumors and could function as oncogenes. These proteins might become the efficient therapeutic targets in treatment-resistant OV tumors.

Alisertib (MLN8237), AT9283, and CYC116 in our protein-drug network classified in kinase inhibitors category. These anticancer compounds are able to inhibit AURKA protein. On the other hand, cyclin-dependent kinases (CDK1) could be inhibited by Flavopiridol, Alsterpaullone, SU9516, AT7519, Olomoucine, Hymenialdisine, and indirubine-3'-monoxime.

In this study, we conducted a novel regulatory network of TF-miRNA-gene interactions in OV tumors. It reveals the role of regulatory feedback and feedforward loops in great complexity of OV tumors. This network indicates that TFs TFAP2C and GATA4 have the most interactions and are involved in feedback loops with miR-26b. According to Cai et al report, GATA4 has lost its expression in ovarian tissues during cellular transformation and progression to malignancy.<sup>73</sup> In our study, for the first time, TFAP2C has been identified to be an important TF in ovarian cancer and might become both biomarker and therapeutic target. Interestingly, the expression level of

miR-26b in patients with ovarian cancer shows a significant reduction and correlates with the stage of tumors and patient's life expectancy.<sup>74</sup>

In conclusion, in current study, we have collected and integrated different data to uncover the complex molecular interactions and oncomechanisms in OV tumors. The DE miRs-DEGs and dysregulated TF-miRNA-gene network reveal the potential interactions that could be significant pieces of OV tumors onco-puzzle.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, S.K.; *Software*, S.K., F.D.; *Resources*, S.K., F.D.; *Writing - Original Draft*, S.K.; *Writing - Review & Editing*, F.D.; *Supervision*, J.T.-B.; *Project Administration*, J.T.-B.

## ETHICAL STATEMENT

Not applicable.

## DATA AVAILABILITY STATEMENT

The required data are provided in the supplementary tables.

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## SUPPORTING INFORMATION

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