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CDK members and ribosomal proteins, a crucial role players in serous capillary ovarian cancer: Insights from gene expression and protein interaction networks

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Ovarian cancer (OC) is an appalling disease of the female reproductive tract and among the most challenging gynecological malignancies. It is often referred to as the "silent killer" because its signs and symptoms are absent, in most cases, until it reaches advanced stages, wherein there is a low probability to cure it. The number of disease markers that have been detected via genome wide expression profiles has increased vastly. One such impressive and encouraging tool to discover therapeutic targets in this kind of complex disease conditions is microarray gene expression profiling. The study aims to perform a comprehensive analysis of differentially expressed genes (DEGs) and associated biological networks in ovarian cancer using bioinformatics tools. This research aims to examine differentially expressed genes (DEGs) in ovarian cancer using the GSE14407 dataset from the GEO database. It will investigate the biological functions and pathways of these DEGs through GO and KEGG enrichment analyses and build a protein-protein interaction (PPI) network to identify key hub genes involved in ovarian cancer development. Genes expressed differentially in ovarian cancer dataset (GSE14407) retrieved from a GEO database were evaluated by the limma package (R language) and via cluster analysis. The function of the DEGs and their pathway analysis was augmented via gene ontology (GO) and Kyoto Encyclopedia Genes and Genomes (KEGG) database, respectively. Additionally, the protein-protein interaction network of the DEGs was built and the topological metrics of the central network generating clusters of highly correlated genes and the hub genes in the network were evaluated. The total of 2225 Ovarian cancer probes were found in GEO dataset. Gene and pathway enrichment analysis shows that up-regulated DEGs were found to be, cell cycle (15 genes), pathways in cancer (14 genes) and p53 signaling pathway (8 genes), meanwhile the down- regulated DEGs were also enriched in pathways in cancer (55 genes), PI3K-Akt signaling pathway(48genes), proteoglycans in cancer (41 genes) and the hub proteins (12 genes). Our findings showed that AURKA, CCNB1, CDK1, CHEK1, CCNA2, CCNB2 were involved in the cell cycle and p53 signaling pathway and their critical role in ovarian cancer. The findings of the present study provide a comprehensive bioinformatics analysis of DEGs that might be involved in pathogenesis of ovarian cancer. The functional network-based analysis that incorporates gene ontology and pathway based information revealed protein kinase as the key player in establishing the ovarian cancer.

Keywords: Cyclin-dependent kinase, Ovarian cancer, Protein kinase, Ribosomal proteins

Ovarian cancer (OC) is an appalling disease of the female reproductive tract and among the most challenging gynecological malignancies. It is often referred to as the "silent killer" because its signs and symptoms are absents, in most cases, until it reaches advanced stages, wherein there is a low probability to cure it. The diagnosis of almost 70% of OCs occurs in the advanced stage, whereas around 35% of the patients tend to survive at 5 years, while the mortality rate tends to remain unchanged for a longer duration[1]. The development of epithelial ovarian cancers is a multiphasic and a multi factor process with different genetic and epigenetic alterations. The major molecular factor involved in ovarian cancer biology is focused on unregulated proliferation (p^{27} kip1 Ras-MAPK, PTEN/PI3K-AKT), apoptosis inhibition/ survival (p53, hTERT) genetic instability, invasion and angiogenesis (MMP-2, MMP-9, VEGF, bFGF). The cyclin dependent kinase inhibitors (CDK) and the expression of p21 are lost in nearly 25-75% of ovarian cancers. Mutated R as genes have been found in 20-25% of OCs[2]. ThePI3K-AKT pathway is the intracellular cascade that is frequently deregulated in OCs, while the AKT inactivation was reported at around 70%and PI3K activation was detected at level of 30%[3]. The tumor-suppress or genes that have akeyrolein OC establishment and development are altered in nearly 30-80% of the OCs studied by

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numerous scientific communities worldwide[4]. Therefore, the disease's clinical significance, its underlying mechanisms and its onset and progression tend to remain in the category of most poorly understood ovarian cancer out of all the human cancers. The various mechanisms and pathways involved in the progression of the tumor are highly intertwined such that most molecular markers falling under a particular category have a role in different aspects of ovarian cancer biology. As such, a more comprehensive analysis of OC biomarkers, with special attention on those molecules that possess well-defined prognostics or/and therapeutic implications, will be undertaken.

The stage of the OC plays a key role in its treatment. Surgery as a treatment can be considered when the ovarian cancer is organ-confined but any progression beyond the ovaries will require a combination of chemotherapy and surgery[5]. Carboplatin and paclitaxel are the crucial drugs that used in the chemotherapy treatment of OC. Currently, one of the biggest challenges linked with chemotherapy is the fact that cancers have begun developing resistance against these drugs and, thus, their effectiveness in eliminating the tumors has diminished significantly[6]. A plethora of contributions provided by numerous studies have helped to research the therapeutic methods of OC[7].

In recent times, the number of disease markers that have been detected via genome wide expression profiles has increased vastly. One such impressive and encouraging tool to discover therapeutic targets in these kinds of complex disease conditions is micro arraygene expression profiling[8]. The findings from this research are most likely to play a key role in OC establishment and can also serve as biomarkers in the diagnosis and treatment of OC.

Materials and Methods

Materials

Affymetrix microarray gene expression data import

The gene expression profile of GSE14407 was downloaded from the NCBI-Gene Expression Omnibus database[9] based on Affymetrix Human Genome version, U1332.10. The analysis was performed on 24 human ovarian samples, 12 of which were normal ovarian surface epithelial cells and the remaining 12 were laser-captured micro-dissected severe capillary OCs. The CEL files of the Affymetrix platform and the probe annotation files originate from the GPL570 platform.

DEG screening

The actual CEL expression profiling records were pre-processed by means of the GCRMA (Gene Chip Robust Multi Array Algorithm) method[10] implemented in Affymetrix micro arrays package of R/ Bioconductor[11] followed by background correction. The probes mapping with more than one gene and the probes without gene annotation were discarded from the list. The false discovery rate (FDR) was used to quantify the expected proportion of false positives among all the DEGs and cancerous ovarian cells. The t-test was performed *via* limma package[12]. In order to reduce the false positive rate of occurrence in the data, an adjusted p value was applied according to the methods devised by Benjamini and Hochberg[13]. Accordingly, adjacent $P< 0.05$ and $|\log FC| >2$ was set as the threshold criterion for the DEGs selection.

Gene enrichment analysis

The recognition of the biological processes, molecular function, and the cellular localization of comprehensive transcriptome data is aided by gene ontology analysis[14]. The Database for Annotation, Visualization and Integrated Discovery (DAVID) web server was employed for the functional annotation of DEGs AMax. prob<0.1 and Min. count>2 and FDR <0.05 threshold criteria were used to identify the gene ontology. The Kyoto Encyclopedia of Genes and Genomes (KEGG) was utilized to determine the significant pathways for the initially screened gene data set[15]. A count of >2 and EASE 0.05 was set as thecut-off criteria for the KEGG pathway analysis.

PPI network construction

To identify the ideal proteins, their interactions and the molecular mechanism of the disease, a protein-protein interaction (PPI) network was established. String (Search Tool for the Retrieval of Interacting Genes) database was used to provide the interacting and experimental information about the proteins encoded by the DEGs[16]. A confidences core value of >0.4 was used as the cut off criteria. The protein-protein interaction network of up-regulated and down-regulated DEGs was visualized using Cytoscape[17].

Computation of topological parameters and identification of hub genes

The PPI network was characterized by topological parameters such as the degree of distribution of proteins, the average number of neighbors, the network density and the clustering coefficients by the

means of Network Analyzer plugin of Cytoscape[18]. The limited number of proteins in the network were highly interconnected and hence indicated that they interact with many other proteins. These proteins are called hub proteins and they play a key role in the different biological processes. In our PPI network, we identified the hub proteins using the CytoHubba plugin[19] based on degree, closeness, Maximal Clique Centrality (MCC) and betweenness measures.

Network module generation and visualization

The constructed PPI network is large and it is less substantial to extract more specific information. Hence, it was necessary to separate the network into sub-network. Each sub- network represented a module. The network modules were acquired based on Molecular Complex Detection (MCODE) plugin of Cytoscape. The parameters with a node score cut-off of 0.2, K score of 2, Max depth of 100 were fixed as the criteria for screening the network modules. The modules were then validated by subjecting them to enrichment analysis which involves spotting out the biological process, cellular components and molecular function nomenclatures with a threshold p value of < 0.05 using DAVID server. ClueGO[20], a Cytoscape plug-in, was used to envision the functionally grouped terminologies modelled as networks. Finally, we analyzed the association of the terms and the functional groups in the biological network based on the kappa score level >4 using ClueGO[21].

Results and Discussion

To identify DEGs between OSE and CEPI**,** the data is processed before and after normalization. After normalization, none of the samples stood out from the rest although the different arrays have very comparable median expression levels as shown (Fig. 1A). Transcriptome analysis was performed between ovarian normal surface epithelial samples and serous ovarian cancer epithelial samples, and after pre-processing and normalization, a total of 2225 probes were collected (Table 1). The cut-off criteria were used as a basis to screen a total of 310 up-regulated and 1885 down-regulated genes.

We noticed that many of the DEGs were already reported to be involved in ovarian cancer. We then sorted and filtered the top three up-regulated genes, which were, ZIC1, ASOX17 and CD24 while the top three down-regulated genes were ZFX, ANXA7, and PRDX1. A Box plot were used to represent the gene expression levels of the DEGs in all the samples (Fig. 1B).

Gene and pathway enrichment analysis

The molecular function, the biological process and the cellular component analysis were performed separately for the differentially expressed up-regulated and down-regulated genes. It is observed that the set of supplied genes are significantly enriched in ovarian cancer. A snapshot of these results with higher gene count and lesser FDR is presented in (Table 1).

The enriched function of the up-regulated DEGs was mainly involved in the cellular process, nuclear division, binding and protein binding, while the down-regulated genes were enriched in the regulation of the biological process, localization, cell part, cytoplasm, cell-cell adhesion, protein binding and calcium ion binding. We noticed that the overall gene patterns were participating in processes such as nuclear division, cytoplasm, cellular components, cell part and protein binding. KEGG

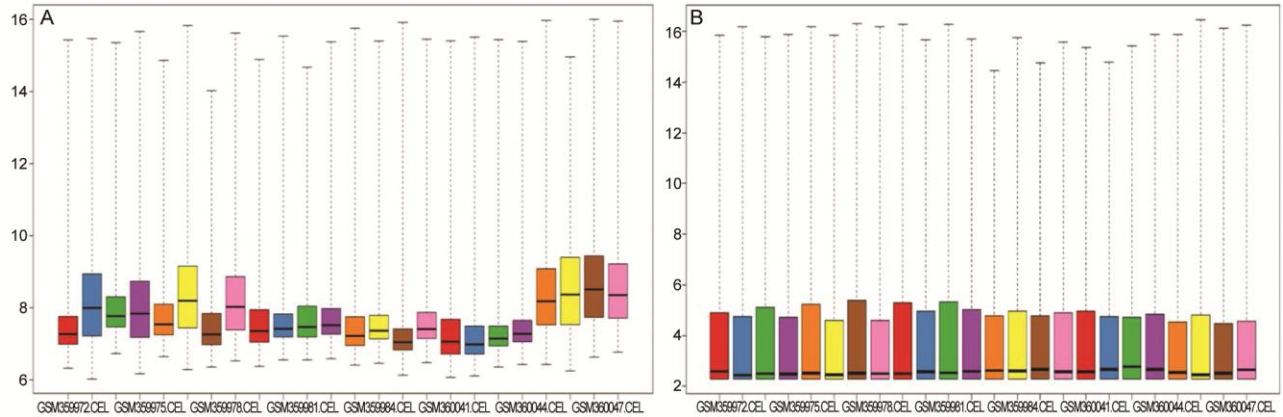

Fig. 1 — Normalization and histogram of Differentially expressed genes (DEGs) between ovarian surface epithelia (OSE) and laser-captured micro-dissected serious capillary OCs (CEPI). (A) Overall expression level of probes intensity values; (B) box plots of normalization probes show similar width and positions

Table 1 — Higher enriched terms of up and down-regulated genes

Gene Xpression	Term	Genes
Up-regulated	Cell cycle GO: 0007049	BUB1B, CTCFL, CKS2, DLGAP5, E2F7, E2F8, FANCD2, NEK2, NUF2, RACGAP1, SAC3D1, SPC24, SPC25, TPX2, ZWINT, ASPM, AURKA, CHEK1, CDCA3, CDCA5, CDCA7, CENPF, CEP55, CDT1, CCNA2, CCNB1, CCNB2, CCNE1, CDK1, CDKN2A, CDKN3, ECT2, FAM83D, HELLS, HMGA2, KIF18B, KIF23, IF2C, KNL1, LZTS1, MKI67, MELK, MCM2, MCM4, NCAPG.
Down-regulated	Cell- division GO: 0051301	BUB1B, CKS2, NEK2, NUF2, RACGAP1, SAC3D1, SPC24, SPC25 TPX2, ZWINT, ASPM, AURKA, BIRC5, CDCA3, CDCA5, CDCA37, CENPF, CEP55, CCNA2, CCNB1, CCNB2, CCNE1, CDK1, ECT2, FAM83D, HELLS, HMGA2, KIF18B, KIF23, KIF2C, KNL1, NCAPG, NUSAP1, PTTG1, PSRC1, PRC1, SPAG5, TIMELESS, UBE2C.
	Chromosome GO: 0005694	BUB1B, POLQ, NEK2, NUF2, SPC24, SPC25, ZWINT, BIRC5, CDCA5, CENPA, CENPF, CENPK, CENPO, CENPU, DTL, HMGA1, HIST1H1C, HIST1H3A, HIST1H4H, KIF2C, KIF4A, KNL1, MKI67, NCAPG, NUSAP1, SPAG5.
	Nucleoplasm GO: 0005654	BCL11A, BLM, CXXC5, DEPDC1, POLQ, E2F7, EHF, FANCD2, GINS1, GINS2, KIAA0101, MECOM, POU2F3, RAD51AP1, RACGAP1, RMI2, SOX17, SOX9, TPX2, ZIC1, AURKA, BIRC5, CDCA5, CDCA7, CENPA, CENPF, CENPK, CENPO, CENPU, CHEK1, CDT1, CBX2, C2ORF88, CCNA2, CCNB1, CCNB2, CCNE1, CDK1, CDKN2A, DTL, EZH2, ESRP1, EME1, GDF11, HMGA1, HMGA2, HIST1H3A, HIST1H4H, KIF23, KIF4A, KNL1, MCM2,
	Cell-Cell adhesion GO: 0098609	DHX29, IQGAP1, LIMA1, LRRFIP1, PDLIM5, ARHGAP18, SH3GLB1, SLK, SWAP70, USO1, VAPA, ANXA2, BZW1, CALD1, CAST, HMP2B, COBLL1, EIF3E, EIF5, FLRT3,
	Cadherin binding involved in cell-cell adhesion GO: 0098641	DHX29, IQGAP1, LIMA1, LRRFIP1, PDLIM5,
	Cell-Cell adherens junction GO: 0005913	ARHGAP18, SH3GLB1, SLK, SWAP70, USO1, VAPA, ANXA1, ANXA2, BZW1, CALD1, CAST, CHMP2B, COBLL1, EIF3E, EIF5, EZR, HSP90AB1, HSPA8, KTN1, KIF5B, PARVA, PRDX1, PKP2, PHLDB2, PKN2, RDX, RACK1, RTN4, RSL1D1, RPL14, RPL15, RPL6, SEPT7, SNX1, SNX2, SPTBN1, SDCBP, YWHAB.
	Calcium-dependent phospholipid binding GO: 0005544	ANXA1, ANXA2, ANXA3, ANXA4, ANXA5, ANXA8, CPNE3, MCTP2, PLA2G4A, PCLO, SYT1, SYT4, SYTL4, ANXA6, ANXA7
		CD200, DHX29, QGAP1, LIMA1, LRRFIP1, PDLIM5, ARHGAP18, SH3GLB1, SSX2IP, SLK, SWAP70, USO1, VAPA, ANXA1, ANXA2, BZW1, CALD1, CAST, CHMP2B, COBLL1, EIF3E, EIF5, EZR, HSP90AB1, HSPA8, KTN1, KIF5B, PARVA, PRDX1, PKP2, PHLDB2, PKN2, RDX, RACK1, RTN4, RSL1D1, RPL14, RPL15, RPL6, SEPT7, SNX1, SNX2, SPTBN1, SDCBP, YWHAB.

analysis revealed the pathways for the differentially expressed up-regulated and down-regulated genes which are presented in (Fig. 2).

The up-regulated DEGs were found to be enriched in three pathways: cell cycle (15 genes), pathways in cancer (14 genes) and p53 signaling pathway (8 genes), meanwhile the down- regulated genes were also enriched in three pathways: pathways in cancer (55 genes), PI3K-Akt signaling pathway (48 genes) and proteoglycans in cancer (41 genes) are shown in (Table 2).

Construction of protein-protein interaction network

String database was used to construct the PPI network and noteworthy interactions were screened with a score greater than 0.8. The PPI map was constructed for top enriched function and pathways of differentially expressed up and down-regulated genes shown in (Fig. 3).

The interaction of protein pairs with up and down-regulated genes consists of 130 nodes, 1876 edges and 218 nodes, 1183 edges, respectively; the topological coefficient confirms the tendency of the nodes into shared neighbors with others and the up and down-regulated DEG's topological parameters as shown in (Table 3 and Fig. 4). The average number of degrees and clustering coefficient of up and down DEG genes were 28.9 10.9 and 0.681, 0.4683, respectively. Up-regulated genes with the highest degree and the connected component indicated that the up-regulated genes have more connectivity than the other genes.

The shortest average path length of up and down-regulated genes was around 94% and the network diameter was 7 and 6, respectively.

The average number of differentially expressed up-regulated genes was 33.802 and while that of down-regulated genes was 12.362. This indicated that the up-regulated genes were highly interconnected and were closely related to each other.

Connectivity degree and hub protein analysis

The number of partner proteins interacting with each and every protein is measured by the proteins' degree of distribution. Its basis lies in the power-law which proves that the networks possess scale-free properties. The Cytoscape MCODE plugin was used to calculate the average degree of each node in the network. Higher connectivity degree of up and down-regulated proteins are listed in the (Table 4).

The hub proteins were filtered on the basis of the highest average degree, closeness, radiality, and betweenness properties. MCM2, CDK1, TOP2A, BUB1B, ZWINT, AURKA, CCNA2, CHEK1, RPS6, MAPK1, PRCKA, KDR, PIK3R1 were the top hub

Table 2 — Enrich KEGG pathways in up and down-regulated genes

Gene Expression	Pathways	Genes	P-Value
Up-regulated genes	Cell cycle	BUB1B, TTK, CHEK1, CCNA2, CCNB1, CCNB2, CCNE1, CDK1, CDKN2A, ESPL1, MCM2, MCM4,	2.2E-10
Down-regulated genes	p53 signalling pathways	CHEK1, CCNB1, CCNB2, CCNE1, CDK1, CDKN2A, RRM2, SFN	1.6E-5
	Pathways in cancer	CXCR4, CKS2, GNAS, MECOM, BIRC5, COL4A1, COL4A2, CCNE1, CDKN2A, FGF18, FZD10, PAX8, SLC2A1, VEGFA	9.3E-4
	Fanconi anaemia pathway	BLM, FANCD2, RMI2, EME1	2.7E-2
	PI3K-Akt signalling pathway	AKT3, BCL2, SGK3, GNB4, GNG11, GNG12, GNG2, JAK1, KITLG, MET, PHLPP2, REB3L2, COL3A, COL4A5, COL4A6, CHUK, CCND2, CDKN1B, EFNA5, FGF1, FGF13, FGF7, FGF9, GHR, HSP90AB1, ITGA2, ITGAV, ITGB5, KDR, LAMA2, MAPK,	1.6E-4
	Ras signalling pathway	PDGFRA, PRKCA, PKN2, PPP2CB, PPP2R5E, PPP2R2B, RAC1, RPS6, SGK1, YWHAB, AKT3, ETS2, GNB4, GNG11, GNG12, GNG2, GAB1, KITLG, MET, TIAM1, CALM1, CHUK, EFNA5, FGF1, FGF13, FGF7, FGF9, GRIN2A, KDR, MAPK1,.	1.9E-3
	Proteoglycans in cancer	AKT3, CD44, FAS, GAB1, IQGAP1, MET, NANOG, CAMK2D ROCK1, ROCK2, TIAM1, TIMP3, WNT16, CAMK2D, DCN, EZR, FLNC, FZD6, FZD7, HPSE, ITPR1, ITGA2, ITGAV, ITGB5, KDR, MAPK1, PIK3R1, PLCE1	3.4E-8
	Rap1 signalling pathway	AKT3, GNAI1, KITLG, MET, TIAM1, CALM1, DOCK4, EFNA5, FGF1, FGF7, FGF9, GRIN2A, KDR, LPAR5, PLCB1,, MAGI2, MAPK1, PIK3R1,, PLCE1, PGDFC, PDGFD, PGDFRA, PRKCA, PRKD1, PRKD3, RAC1	1.6E-2

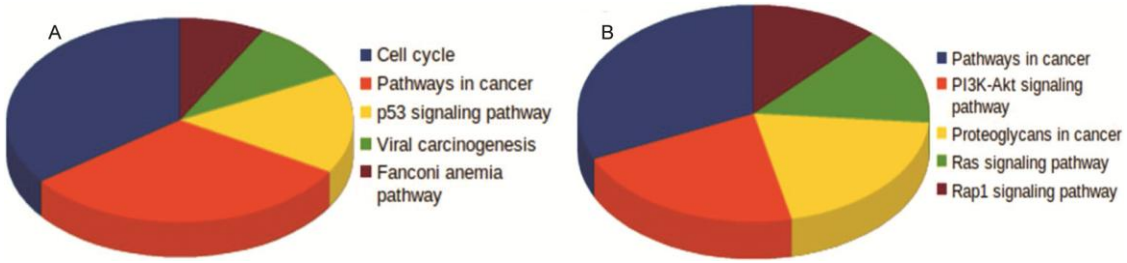

Fig. 2 — KEGG pathway impacted by differentially expressed genes (DEGs). (A) Enriched pathways of up-regulated genes; and (B) Enriched pathways of down-regulated genes

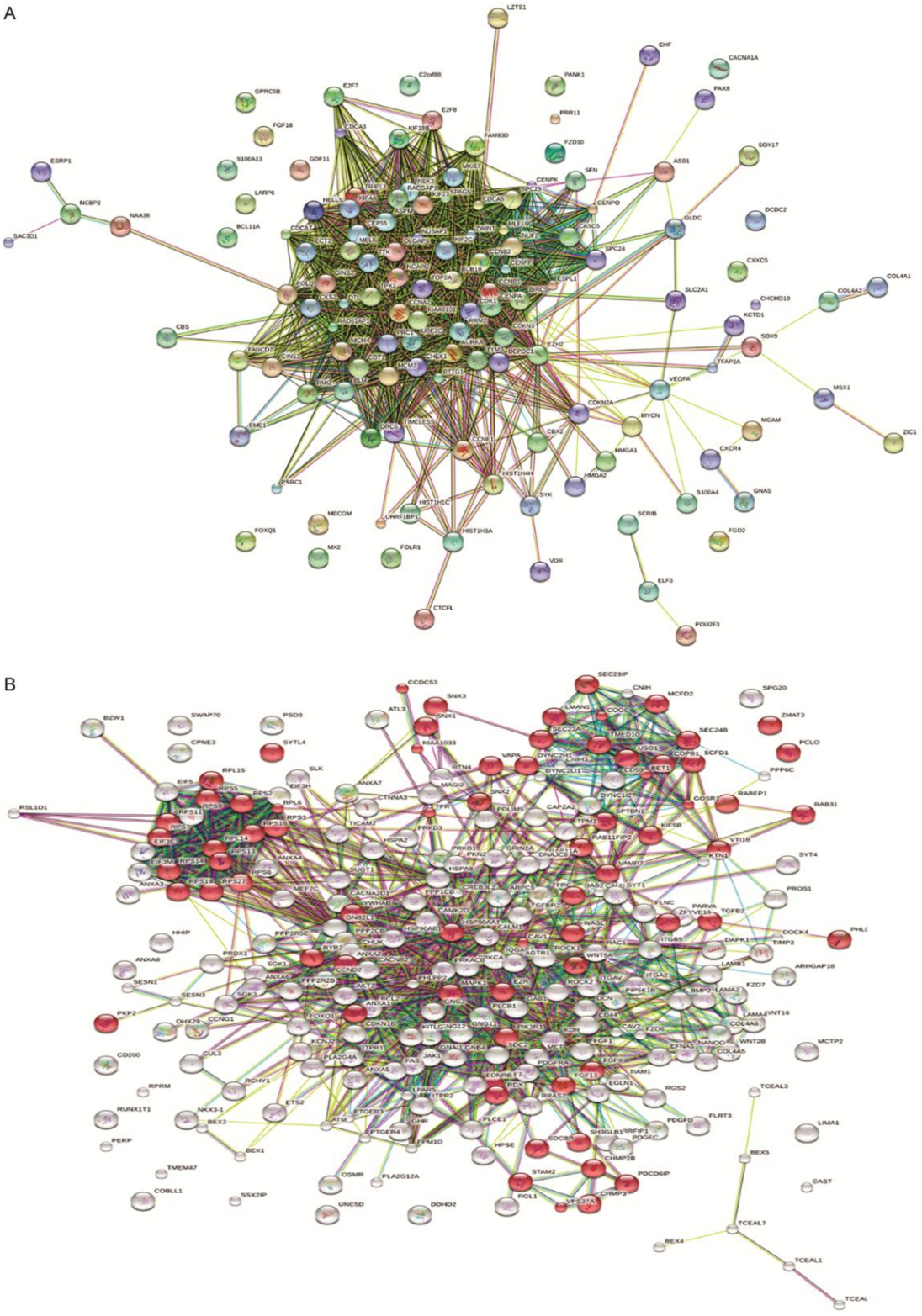

Fig. 3 — Constructed protein-protein interaction network of differentially expressed genes. The node stands for DEGs and lines represent the interaction between DEGs genes. (A) Up-regulated differentially expressed genes network; and (B) Down-regulated differentially expressed genes network

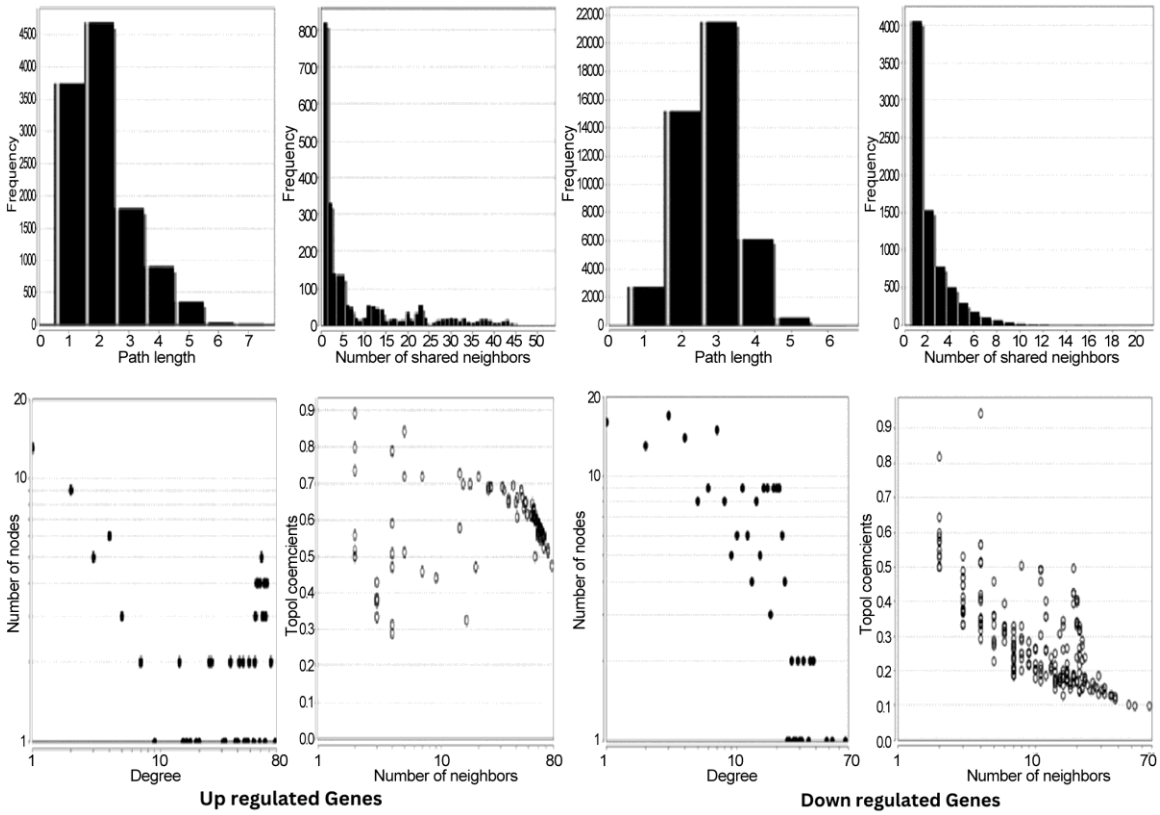

Fig. 4 — The topological parameter interaction network of differentially expressed genes

Table 3— Topological parameters values of Networks

Network	Parameters	Values
Up regulated	Clustering coefficient	0.681
	Network diameter	7
	Network centralization	0.409
	Shortest path	11562 (94%)
	Avg. number of neighbours	33.802
	Network Density	0.307
Down regulated	Clustering coefficient	0.442
	Network diameter	6
	Network centralization	0.251
	Shortest paths	46040 (94%)
	Avg. number of neighbours	12.362
	Network density	0.056

proteins in up and down-regulated networks. These hub proteins are listed in a (Table 5). These proteins play a significant role in maintenance of the global network architecture and communication. CDK1 and MAPK1 were seen to be the hub proteins, having the highest degrees of 78 and 67, respectively. The cyclin dependent kinase and map kinase proteins were the most closely related proteins in up and down-regulated interaction networks.

Table 4 — Top connectivity MCODE degree in up and down-regulated protein-protein interaction

Gene expression	Gene Name	Degree
Up regulated	CDK	178
	CCNB1	72
	TOP2A	72
	BUB1B	67
	AURKA	67
	CCNB2	67
	TTK	66
	CHEK1	66
Down regulated	MAPK1	67
	HSP90AA	153
	CALM1	48
	KDR	38
	PRKCA	38
	BCL	236
	CD44	35
	HSPA8	32
	PIK3R1	31

Sub network modules and functional enrichment analysis

The sub-network modules of up and down-regulated genes with the highly interconnected sub networks modules were raised from both up and down-regulated network hub protein, based on parameters like nodes >6 and high M code score >3

value. These values were considered as roots for clustering as represented in (Fig. 5). A sub-network module from an up-regulated network was derived with a D1 score of 60.831 and was comprised of 66 nodes and 1977 edges. CDK1, CCNB1, KIF23, TTK, and UBE2C were seen as highly interconnected genes. Two sub-networks were derived from a down-regulated PPI network. The first module had a D1 score of 19.789 and comprised of 20 nodes and 188 edges. These modules had a D2 score of 12.37, connected with 28 nodes.

We noticed that in module (D1), Ribosomal protein L15 (RPL15) was tightly interconnected with the Ribosomal Protein S2 (RPS2), Ribosomal protein L6 (RPL6), Ribosomal Protein S14 (RPS14), Ribosomal Protein S11 (RPS11) and Ribosomal Protein S13 (RPS13). The nodes of the network were annotated in order to gain a full view of the network's crucial function. The gene ontology, biological process, and pathways analysis of up and down DEGs terms were evaluated and have been listed in (Tables 2 and 3). The genes of the up-regulated module are known to take part in the cell cycleprocess, nuclear division, and chromosome organization. Most of the genes were observed to be enriched in the cell cycle process and nuclear division. The down-regulated module genes were also enriched in the cytosolic ribosome, intracellular ribonucleoprotein, cytosolic ribosome, organism's metabolic processes, protein transport, protein localization, and Golgi vesicle-mediated transport with the highest number of genes being involved in ribosome and the organism's metabolic processes.

Table 5 — Hub protein of up and down-regulated networks

Network	Hub Protein
Up-regulated gene	MCM2, TOP2A, NCAPG, BUB1B, ZWINT, AURKA, PRC1, CCNA2, CHEK1, CCNB2, NUSAP1, BIRC5, TTK, KIF2C
Down-regulated gene	AGTR1, BCL2, CALM1, GNB2L1, RPS6 HSP90AA1, KDR, PIK3R1, PRKACB, MAPK1, PRKCA, RAC1, WNT5A

Visualization and interpretation of sub-network enriched modules

After the enrichment, sub-network DEGs were then subjected to GO term and KEGG pathway analysis, which facilitated visualization of the functionally grouped terms in the sub-network presented in (Fig. 6). The bar chart represents the genes associated with the term, hence we observed that the DEGs are enriched in Histone Kinase activity, Protein Kinase and Cyclin Dependent Protein Serine/Threonine Kinase activity. The percentage of associated genes present in the Histone Kinase and Protein Kinase are 23.5% and 12.9%, respectively. The protein kinases were linked by two pathways, namely the oocyte meiosis pathway and the p53 signaling pathway. The up-regulated module was utilized for the purpose of exploratory analysis of function and dysregulated pathways in disease, as exemplified by the highest p-value and percentage of associated genes, and are related to pathways such as the p53 signaling and cell

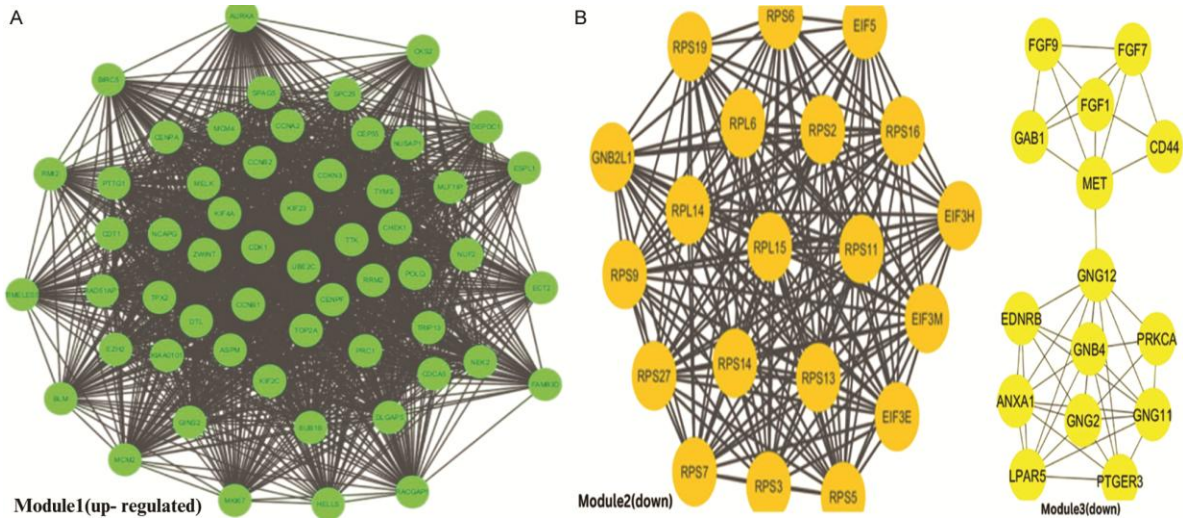

Fig. 5 — Sub network modules of differentially expressed genes. (A) Module1 (up) interaction Network for up-regulated genes represented in green; and (B) Module2 (d1) (d2) interaction networks of down-regulated genes represented in yellow

cycle pathways. It was observed that the up-regulated DEGs module enriched these pathways. The sub-network for the down-regulated module have been shown in (Fig. 7).

The sub-network module differentially expressed down-regulated genes were enriched in rRNA binding and phosphatidylinositol-4-5-bis phosphate 3-kinase activity. The percentage of associated genes present in

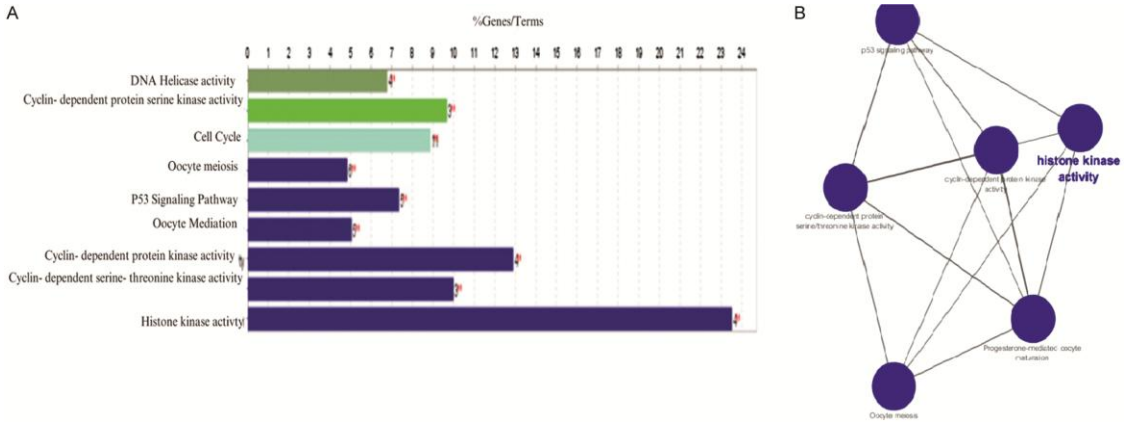

Fig. 6 — The GO-immune system processes molecular function terms and KEGG pathway analysis of up-regulated genes. (A) The bars represent the number of genes and percentage of genes associated with the terms; and (B) The size of the nodes linked is based on their kappa score level (>4) of the term. Functionally grouped network includes genes that are shared between different KEGG pathways. The colors represent the enrichment significance-the deeper the colorona color scale, the higher the enrichment terms. P-values were adjusted using a Benjamin and Hochberg False Discovery Rate (FDR) correction

Fig. 7 — GO-molecular function terms and KEGG pathways analysis of down-regulated genes.The size of the no deslinked is based on their kappa score level (>4) of the term. Functionally grouped networks include genes that are shared between different KEGG pathways.Thecolors represent the enrichment significance the deeper the colorona color scale, the higher the enrichment terms. P-values were adjusted using a Benjamin and Hochberg False Discovery Rate (FDR) correction

these is 9.09 and 6.94. We observed that the number and percentage of genes were enriched in pathways such as the Ras signaling pathway and ribosome. It has been well established that the dysregulation of cell cycle is one of the hallmarks of several cancers, including ovarian cancer[22]. Mechanisms of regulation of the cell cycle primarily include cyclins, cyclin-dependent kinases (CDK) and CDK inhibitors. An uncontrolled cell proliferation is seen as a result of the alterations of these mechanisms and is a distinguishing facet of human cancers. It is a well-known fact that the cell cycle plays a role in the etiology of spontaneous cancers[23]. P53 protein, directly and indirectly regulates the transcription of the genes involved in the cell cycle and apoptosis, due to which it can inhibit malignant transformation[24]. TP53 has the highest frequency of undergoing mutations in human cancer[25]. Our findings showed that AURKA, CCNB1, CDK1, CHEK1, CCNA2, and CCNB2 were majorly involved in the cell cycle and p53 signaling pathway, and were hub genes in both PPI Network and sub-network, which highlighted their critical role in ovarian cancer.

Cyclin-dependent kinases (CDKs), which are serine/threonine protein kinase family members have been shown to play critical roles in the proliferation of tumor cells and their growth as they regulate transcription, cell cycle, and RNA splicing[26]. Universal over activity of the cell cycle CDKs in human cancers, and their inhibition can cause both cell cycle arrest and apoptosis. There is a genetic deregulation of the CDKs that are involved in the coordination of the S phase. CDK1 activity is dysregulated due to direct genetic alterations in tumor genesis[27] and occurs indirectly as a result of faulty p53 signaling or impaired DNA damage checkpoints. The overexpression of CDK1 has been associated with lung cancer, lymphoma, and advanced melanoma. A high cyclin B1 expression usually implies a more aggressive cancer phenotype. Cyclin B1, which is a regulatory protein, is involved in mitosis and has an essential part to play in cell proliferation. CCNB1 expression level is low in the case of normal tissues but was overexpressed in tumors containing mutation in TP53[28]. The overexpression of CCNB1and/or its mis-localization has been reported in many primary cancers such as colon, breast, thyroid carcinoma, prostate, gastric, and non-small-cell lung cancer[29].

Aurora kinase A (AURKA) plays a crucial role in mitotic entry, bipolar spindle assembly and centrosome function. AURKA has been involved in G2/M transition regulation; G2/M arrest and apoptosis occur as a result of AURKA expression inhibition, while cells are allowed to bypass the G2/M DNA damage checkpoint due to its ectopic expression[30]. AURKA takes part in p53 regulation (p53 being a notable tumor suppressor) by phosphorylating both Ser315 and Ser215 residues[31]. This causes increased degradation of p53, and inhibition of p53 transcriptional activity (mediated by Mdm2), respectively. Intriguingly, AURKA is also involved in inhibition of p53 activity through phosphorylation of hnRNPK (heterogeneous nuclear ribonucleo protein K) on Ser379, which is p53's transcriptional co activator and is required for activating p53 in case where there is damage to genes[32]. AURKAover expression is a common feature of Epithelial Ovarian cancer and other solid tumors and has been linked to transcriptional activation, delayed protein degradation, or gene amplification[33]. Increased AURKA levels in primary ovarian tumors are linked with supernumerary centrosomes and decreased survival overall[34], which suggests that AURKA has a crucial part to play in ovarian cancer biology. Ribosomal proteins (RPs) belonging to a family of RNA-binding proteins, are essential components of the ribosome. Several recent studies have revealed that RPs have additional extra-ribosomal functions, independent of protein biosynthesis, in the regulation of diverse cellular processes[35]. The role of ribosomal proteins as tumor suppressors has been observed by somatic or germ line mutation identification and these occur in some RP genes in several cancers seen in humans. RPs play major roles in regulating the progression of the cell cycle through mechanisms that are p53-dependent and independent. It is remarkable that several RPs have been shown to act as activators of p53 and inducers of p53-dependent cell cycle arrest along with apoptosis due to ribosomal stress, and these includeL5, L6, L11, L23, L26, L37, S3, S7, S14, S15, S20, S25, S26, S27, and S27L[36]. This occurs because RPs bind to MDM2, which causes inhibition of p53 ubiquitylation and degradation (mediated by MDM2), ultimately causing activation of p53[37]. As a response to extracellular stress, the Ribosomal protein S3 induces apoptosis via activationof JNKs (c-Jun N-terminal kinases), with the process being caspase dependent[38]. Some RPs, like S27 and L23, have been shown to possess anti-apoptotic activity. S27 (also called metallopanstimulin-1 or MPS-1) was seen to be highly expressed in several human cancers. Apoptosis was induced after its knock-

down as a result of NF-κB activity inhibition occurring due to reduced phosphorylation of p65 at Ser536 and phosphorylation of IκBα at Ser32. Apoptosis, DNA repair and gene transcription are some of the additional extra ribosomal functions of RPS3. Its phosphorylation is regulated by Akt, and can possibly be a factor of paramount importance in neuronal cells as it determines either the pro apoptotic function or DNA repair activity. Along with that, the RPS3 protein has also been shown to take part in apoptotic pathways, as it plays a role in the apoptotic process in NIH3T3 cells.

The phosphatidylinositol 3 Kinase pathway, also called the PI3K pathway, is a complex signaling network. Several direct upstream inputs from growth factors are coordinated by the PI3K pathway. It also coordinates tyrosine kinase receptors and several membrane receptors like Mek and also monitors cross-talk with Ras-Raf- Mek-Erk signaling pathway. PI3K consists of a p85 regulatory subunit and a p110 catalytic subunit. The latter phosphorylates PIP2 (phosphatidylinositol-4, 5-bisphosphate) to PIP3, which is the active second messenger. PIP3 recruits Akt to the plasma membrane, causing a change in conformation and activation of Akt and PDK1 proteins. The former is a serine-threonine kinase and is known to regulate many downstream targets whereas the phosphatase and tensin (PTEN) analog protein acts as an endogenous pathway repressor by causing de-phosphorylation of PIP3, converting it back to PIP2. Akt has control over critical cellular survival as well as metabolic processes[39]. It has been reported that the PI3K/Akt/mTOR pathway undergoes frequent dysregulation in ovarian cancer.

The levels of expression of both pAkt and p110α were analyzed in more than 500 ovarian cancer tumors and were associated with a reduced rate of survival[40]. The pathway's activation, as seen from levels of phosphorylation of Akt or mTOR is almost always seen in ovarian cancers and is an independent negative prognostic marker[41]. The fibroblast growth factor (FGF) along with FGFR (its receptor) axis play significant roles in oncogenesis, however, there is little information on their impact in ovarian cancer. We aimed to determine the relation between genetic variants present in the FGF pathway and the risk, response to therapy, and survival of patients diagnosed with or suffering from ovarian cancer.

Conclusion

In summary, the findings of the present study provide a comprehensive bioinformatics analysis of DEGs that might be involved in pathogenesis of ovarian cancer. The protein-protein interaction network was built with the genes recognized/ identified by gene expression analysis, and it was found that AURKA, CCNB1, CDK1, CHEK1, CCNA2, CCNB2, RPS14 and RPL15 potentially have a direct correlation with ovarian cancer. The functional network-based analysis that incorporates gene ontology and pathway based information revealed protein kinase as the key player in establishing the cancer. These finding could help advance the understanding of molecular mechanisms of ovarian cancer and provide potential therapeutic targets for the development of new treatment avenues.

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Conflict of interest

All authors declare no conflict of interest.

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