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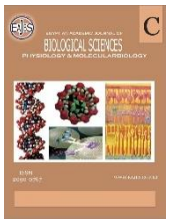

Identification of Ovarian Cancer Using in Silico-Based Analysis of the Downregulated Expressed miRNAs

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ABSTRACT

Ovarian cancer (OC) is one of the top global reasons of death among women with high prevalence. Ovarian cancer can be categorized into epithelial, non-epithelial, and metastatic types. Animal models such as mice are intensively utilized to investigate the molecular mechanism controlling cancer development in the human beings. Recently, several approaches have been extremely studied to control ovarian cancer at the transcriptional or post-transcriptional levels using small RNAs molecules including microRNAs. These molecules have played a key role in the growth of malignant tumour of ovary including cellular proliferation and metastasis. We carried out a meta-analysis of previously published miRNA expression datasets (two human datasets GSE83693 and GSE119055) and one mouse GSE98391 to identify the downregulated miRNA and its target genes with biological processes and pathways. Meta-analysis of miRNA datasets showed that miR-378a-3p, miR-378a -5p and miR-378c are commonly downregulated miRNAs among the three databases in cancerous samples in comparison to normal samples. A total of 405 common gene targets for miR-378a-3p, -5p and miR-378c were identified using miRWALK. Enrichment analysis revealed that miRNAs target genes were predominantly linked to protein binding as well as in Ras signalling pathways. In addition, multiple hub miRNA target genes in the PPI network provided poor prognosis for the patients with OC including FLT1, its level was closely relevant to ovarian cancer. Overall, these investigations exhibited that the defined miRNAs and their target genes could be exploited as biomarkers to identify ovarian malignancies and achieve an early effective therapy.

INTRODUCTION

Ovarian cancer is one of the most outstanding causes of death among women worldwide. There are several types of cancer affecting the ovaries such as epithelial, non-epithelial (germ cell and sex cord-stromal cell), and metastatic usually develop from surrounding cancerous organs (Ricciardi *et al.*, 2018). Epithelial ovarian carcinoma (EOC) is the most prevalent histological type and it can be accounting for about 90 percent of ovarian malignancies (Desai *et al.*, 2014). There are five main subtypes of epithelial ovarian cancer; high and low-grade serous ovarian cancers, endometrioid ovarian carcinoma, ovarian clear cell carcinoma, and mucinous ovarian cancer (T. Guo *et al.*, 2021).

The vast majority subtype of ovarian cancer is high-grade serous ovarian cancer. Whereas, low-grade serous ovarian cancer is less elucidated, represents around 5-10% of the serous type of ovarian cancer, and developed slowly at an early age, it can be identified as genetically stable cancer with a reduced number of mutated genes (Gadducci and Cosio, 2020). Endometrioid carcinoma forms approximately 10–15% of ovarian epithelial carcinomas, reporting as the next most known type of epithelial ovarian cancer and it starts at the middle-aged women 50-60 years and develops from endometriosis. Clear cell cancer of the ovary is a less common subtype composed of 5% of all epithelial ovarian cancers. (Davis *et al.,* 2014). Mucinous ovarian cancer is uncommon subtype of ovarian cancer and made up approximately 3-12% of ovarian malignancies and can be observed in women under the age of 40 (Köbel *et al.,* 2010; Yoshikawa *et al.,* 2014). Most research have utilized rats and mice as animal model to display the stimulatory effects of environmental and internal signals on the growth and metastasis of ovarian tumorigenesis (Choi *et al.,* 2007)

Recently, advances in molecular genetic approaches have revealed a group of small non-coding RNAs, among which are microRNAs (miRNAs). These molecules are highly conserved among species and significantly related to the development of ovarian tissues including cellular proliferation, survival and apoptosis (Mohammed *et al.,* 2017; Sontakke *et al.,* 2014). The possible roles of miRNAs have been demonstrated in several studies as diagnostic biomarkers for ovarian cancers by examine their expression in the cancer tissues (Alshamrani, 2020; Chen *et al.,* 2019; Nam *et al.,* 2008). A recent study has proposed a subset of miRNAs that are predominately expressed in three types of ovarian cancer including high-grade serous ovarian cancer, clear-cell carcinoma of the ovary and ovarian surface epithelium cancer. Some of these miRNAs are comprised of members of miR-200 family and miR-182-5p which are expressed at high levels in both high-grade serous ovarian cancer and ovarian clear-cell adenocarcinoma, whereas miR-383 was notably declined correlated to its expressions in ovarian surface epithelium carcinoma. miR-509-3-5p, miR-509-5p, miR-509-3p, miR-510 and miR-514b-5p distinguished clear cell carcinoma of the ovary from high-grade serous ovarian cancer (Vilming Elgaaen *et al.,* 2014). Moreover, the positive signal of miR-30a-5p and miR-30a-3p found in the clear cell type of ovarian cancer, as well as miR-192 and miR-194 expression was dramatically enriched in the ovarian mucinous tumour (Calura *et al.,* 2013). This study aimed to identify the downregulated miRNAs as well as investigate their functional roles using in silico-based analysis in ovarian cancer patients. A deeper understanding of the molecular regulation involved in the formation of tumour will inform the critical therapeutic approaches to treat ovarian cancer and infertility in animal and human.

MATERIALS AND METHODS

Study Design:

Collection of GEO Datasets:

miRNAs expression datasets were manually explored and obtained from the NCBI GEO database (www.ncbi.nlm.nih.gov/geo), using “the cancer of the ovary or epithelial ovarian cancers” and “miRNAs or microRNAs” as keywords. Two humans, GSE83693, GSE119055 (Dong et al., 2019; Ji Nam *et al.,* 2016) and one mouse GSE98391 (Vuong *et al.,* 2017) were collected. The platform for these experiments in the datasets was GPL22079, GPL21572 and GPL21572, respectively. The selected datasets were chosen according to the following criteria (1) the tissue specimens were obtained from human and mouse ovarian cancer and normal cells; (2) the number of samples in each dataset was 3 or more (Table 1).

Table 1. The ovarian cancer database used in this study**.**

Study	GSE accession	Sample size		Platform	Annotation platform	References
		Control	Cancer			
1	GSE83693	4	7	GPL22079	Agilent-031181 Unrestricted_Human_miRNA_V16.0_ Microarray (miRNA_107_Sep09)	(Ji Nam et al., 2016)
2	GSE119055	3	6	GPL21572	[miRNA-4] Affymetrix Multispecies miRNA-4 Array [ProbeSet ID version]	(Dong et al., 2019)
3	GSE98391	3	3	GPL21572	[miRNA-4] Affymetrix Multispecies miRNA-4 Array [ProbeSet ID version]	(Vuong et al., 2017)

Analysis of the Public Database:

The GEOR2 software is an online tool that compares between two subsets of samples in GEO dataset and was employed to distinguish the downregulated miRNAs between cancer and normal ovarian cells. We carried out the following parameters; Benjamini and Hochberg false discovery rate (FDR). P<0.05 and |log2 (FC)|>1 was inserted as the cut-off criterion. miRNAs with P<0.05 and |log fold change (FC)| >1 were recognised as downregulated expressed (Zhang *et al.*, 2018). To visualize the common downregulated miRNAs between ovarian cancer and normal control cells, a Venn diagram was created utilising a browser-based tool http://bioinformatics. psb. ugent. be/webtools/Venn/.

Putative Targets of Downregulated miRNAs in Ovarian Cancer:

To detect the targeted genes of miR-378a-3p, -5p and miR-378c based on miRNA–gene interactions, an online database, miRWALK 2.0 (http://mirwalk. umm.uni-heidelberg.de/) (Sticht *et al.,* 2018) was processed. Three miRNA-targets tools, including TargetScan, miRDB and miRTarBase., were taken into consideration. The score ≥ 0.95, 3 UTR binding sites and more than 10 pairs were utilized as the critical criteria for the predictive analysis.

Functional Gene and Pathway Enrichment Analysis:

DAVID tool (http://david.abcc. ncifcrf.gov/) (Sherman *et al.,* 2007) was performed to obtain the gene ontology annotation and KEGG pathway analysis of the common target genes of the downregulated miR-378a-3p, miR-378a-5p and miR-378c. The enrichment analysis of GO terms was classified into three groups involving biological process (BP), cellular component (CC), and molecular function (MF). We used P<0.01 as statistically significant results.

Protein-Protein Interaction (PPI) Networks of miRNA Target Genes:

The protein-protein interaction (PPI) networks of the target genes were achieved by GeneMANIA based on physical interactions. GeneMANIA v3.5.2 is an online predicated gene function tool, with several networks obtained from different genomic or proteomic datasets. CytoHubba was utilized to display hub genes in the PPI network as follows: the 10 top Hubba nodes ranked by Maximal Clique Centrality (MCC) and according to their rank degree (Chin *et al.,* 2014). The interaction network of hub genes was established and observed with Cytoscape software (Shannon *et al.,* 2003) (v3.9; www.cytoscape.org/).

Survival Analysis:

Survival analysis of the hub genes was carried out using (Ovarian cancer) in Kaplan–Meier (KM) plotter (https://kmplot. com/analysis/index.php?p=service&cancer= ovar). The online KM plotter categorised ovarian cancer patients into high and low-expression groups by the cut-off values of median gene expression. Multiple hub genes were inserted and KM survival plot for the overall survival (OS) of 614 ovarian cancer patients was analysed. P-values <0.05 were considered to be statistically significant.

Genetic Alteration:

The genetic alteration of the hub genes was validated utilising cBioPortal for cancer genomics, a free bioinformatic approach that assesses the gene alteration in multiple studies from 1892 ovarian cancer patients using the Cancer Genome Atlas (TCGA) and the genotype-tissue,

RESULTS

Identification of downregulated-miRNAs in the ovarian cancer samples compared with normal tissues was carried out using GEOR2 software. Three microarray datasets involved in the present study were obtained from the GEO database as shown in Table 1. According to the threshold of $P<0.05$ and log FC ≥ 1, a total of 63 and 79 downregulated miRNAs were observed between cancerous ovarian tissue and normal counterpart for the GSE83693 and GSE119055 samples, respectively (Supplementary Table 1). Using the mouse database, a significantly 9 downregulated miRNAs were detected (Supplementary table 1). To recognise the molecular mechanisms behind the development of OC, common miRNAs among the three databases were examined by a Venn diagram analysis (Fig. 1, A). As a result, among these downregulated miRNAs, miR-378a-3p and miR-378c were identified to be shared by one human and mouse ovarian cancer samples (GSE119055 vs GSE98391). While miR-378a-5p was common among the three GSE datasets. hsa-miR-378a-3p,-5p and miR-378c are homologous to mmu-miR-378a-3p-5p and mmu-miR-378c, they have the same seed regions (miRbase (v22.1)) (Fig 1, B), as result of which they may target the same target genes.

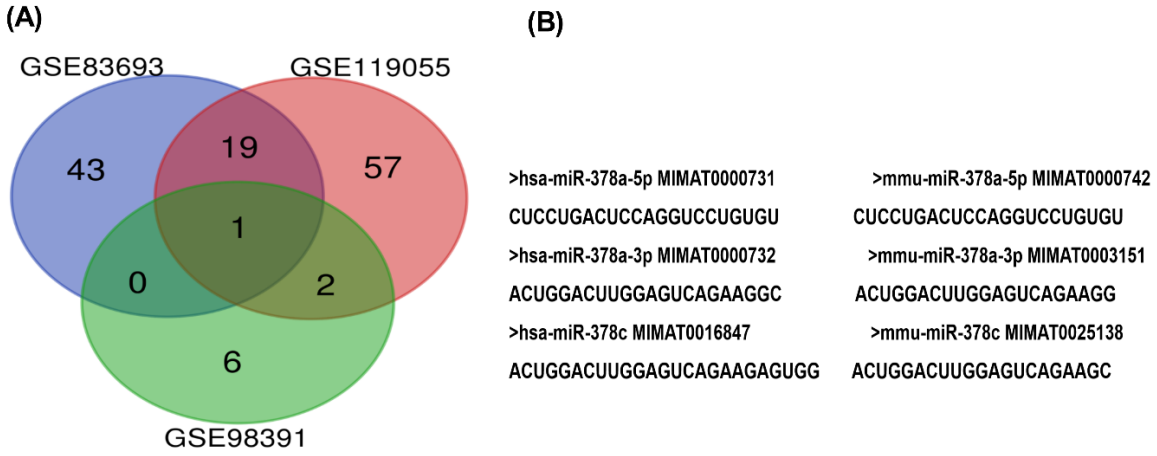

Fig. 1: **(A)** Common downregulated miRNAs between human and mouse databases using Venn diagram http://bioinformatics.psb.ugent.be/webtools/Venn/. **(B).** Mature sequences of miR-378a-3p,-5p and miR-378c in human (has) and mouse (mmu) sourced from miRbase.

Identification of Gene Targets, PPI Network Construction and Gene Ontology Analysis of the Downregulated miRNAs:

We obtained a total of 3426, 3123 and 2600 target genes of miR-378a-3p, miR-378a-5p and, miR-378c, respectively, (Supplementary Table. 2). Of which 405 were common target genes among three miRNAs (Fig. 2). Afterward, the common miRNA target genes were entered into the geneMANIA v3.5.2 database and the interaction network for proteins showed 421 nodes and 7856 edges. The Cytohubba software was utilized to distinguish the highest 20 hub genes involved in the PPI network based on the MCC and degree methods (Fig. 3). The top-ranked hub gene was RBFOX1 followed by ACTR3, DLG2, SMARCA2, CSNK2A1, NR2C2, KIF2A, SRPK1, CDH13 and NTRK2 based on Degree method (Fig. 3, A). RBFOX1 was the most remarkable gene with maximum MCC scores followed by DLG2, MRPS33, FLT1, SMARCA2, CSNK2A1, NR2C2, KIF2A,

IREB2 and CDH13 (Fig. 3, B). The nodes in the interactive network describe as genes, while edges form the protein-protein associations.

DAVID v 6.8 was applied for subsequent gene ontology and KEGG pathway analysis of common miRNA targets. 6 Biological process (BP), 13 cellular components (CC) and 3 molecular functions (MF) terms were engaged as shown in Figures 4, A, B, C. The enriched KEGG items of these hub genes are listed in Figure 4, D. including Ras signaling pathway, Dopaminergic synapse, PI3K-Akt signaling pathway, Focal adhesion and Morphine addiction. Some of the hub genes were significantly enriched in Ras signaling pathway (p<0000007) (Fig. 4, D).

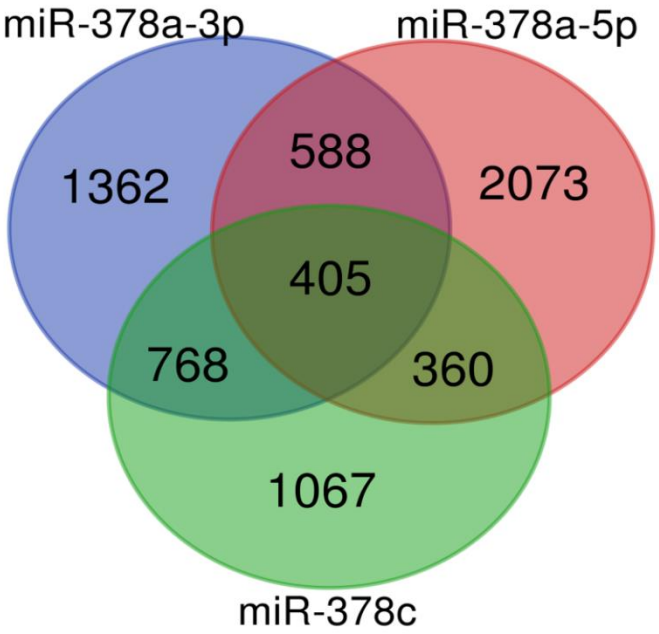

Fig. 2: Common targets of the downregulated miRNAs using miRWALK http://mirwalk.umm.uni-heidelberg.de/ and Venn diagram was generated by http://bioinformatics.psb.ugent.be/webtools/Venn/.

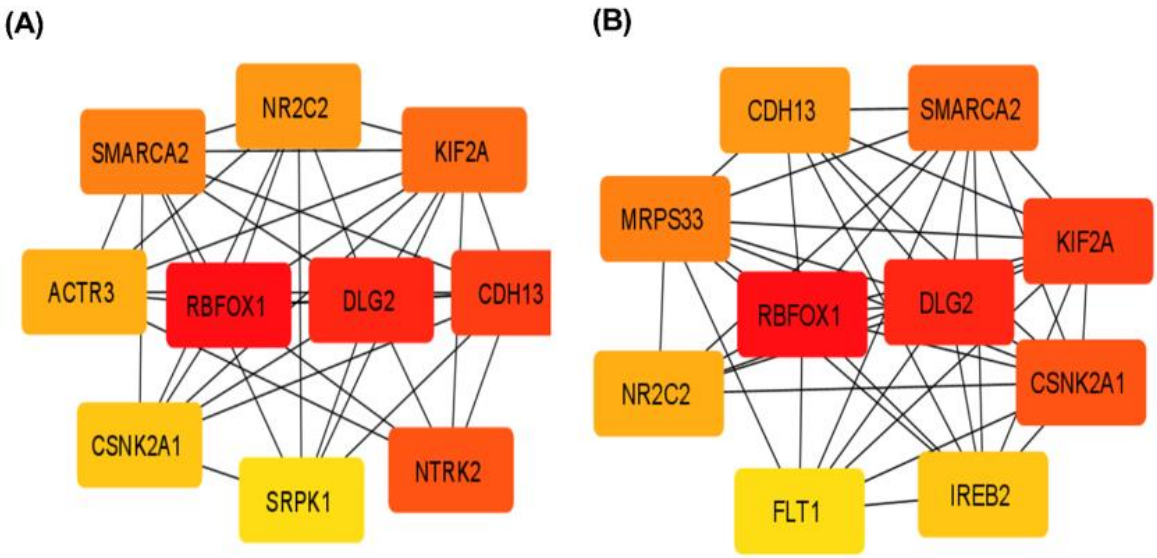

Fig.3: A. B. Top significant subnetwork module was constructed from the PPI network, showing the interaction of miR-378a-3p, 5p and miR-378c target genes using the CytoHubba plugin with highest score and visualized by Cytoscape software.

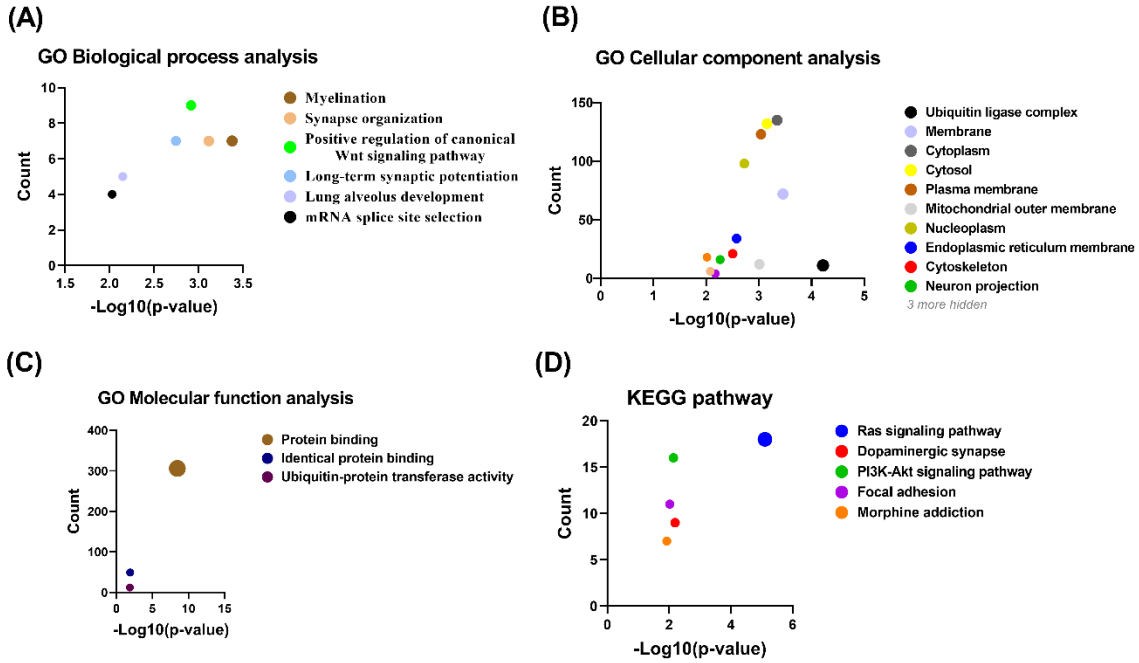

Fig.4 : Functional GO and KEGG terms enriched for target hub genes of miRNAs using DAVID tool. The color represents a GO term and the size represents the term enrichment significance, $p \leq 0.01$. Terms are connected based on shared genes.

Survival Analysis of the Hub Genes:

KM analysis was run on 614 patients to confirm the prognostic importance of the ranked genes in this study. The main findings showed the high expression of 6 of the top hub genes (RBFOX1, DLG2, NR2C2, KIF2A, FLT1 and MRPS33) in PPI network are associated with poor prognosis and suggested that these genes may be used as indicators to monitor the diagnosis of ovarian cancer (Fig. 5).

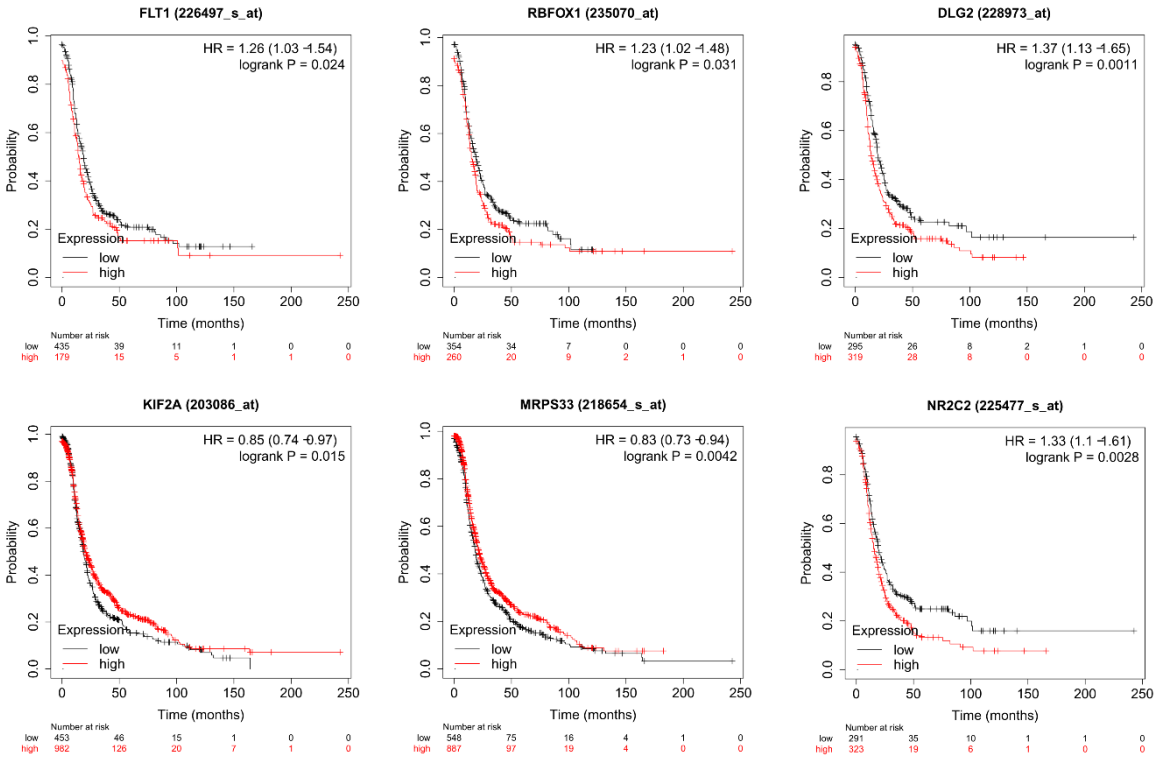

Fig. 5: Survival analysis of the hub genes in the MAPK signalling pathway via KM plotter [p<0.05]; the red line represents high expression, and the dark line represents low expression in OV patients.

Genetic Alteration Analysis of the Hub Genes:

RBFOX1, DLG2, NR2C2, KIF2A, FLT1 and MRPS33 were altered in 209 (26%) samples of 818 patients from four out of seven TCGA databases, including Ovarian Serous Cystadenocarcinoma (TCGA, Firehose Legacy), Ovarian Serous Cystadenocarcinoma (TCGA, Pan Cancer Atlas), Ovarian Serous Cystadenocarcinoma (TCGA, Nature 2011), High-Grade Serous Ovarian Cancer (MSK, 2021) and the alteration rates were 28.94% (of 584 cases), 23.97% (/584 cases), 19.84% (489) and 4.44% (of 45 cases), respectively (Fig. 6A). The top mutated gene was DLG2 with 11% then MRPS33 (8%), RBFOX1(4%), KIF2A (4%), FLT1 (2.2%) and NR2C2 (1.3%) (Fig. 6B).

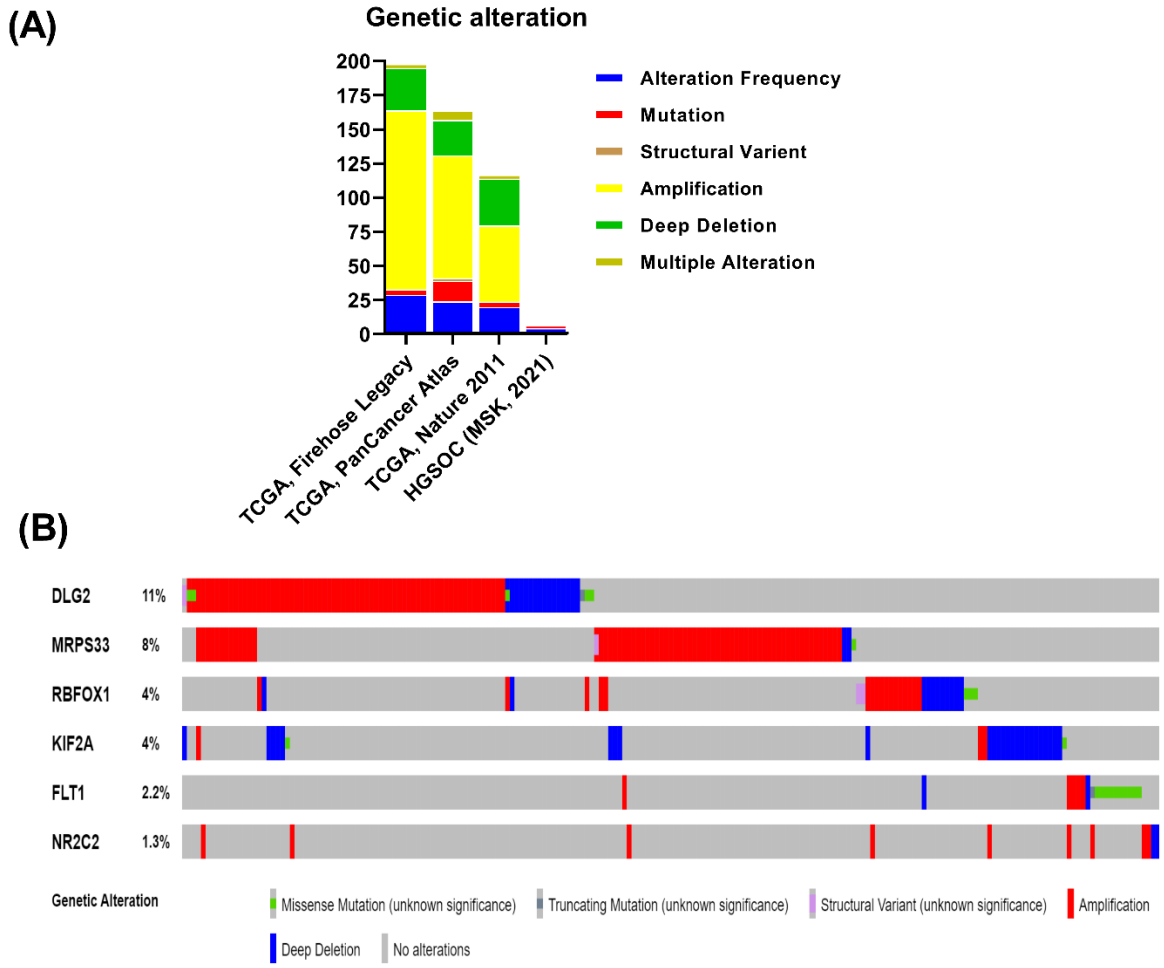

Fig 6: Genetic alteration of the hub genes obtained from Ovarian Serous Cystadenocarcinoma TCGA, Firehose Legacy, Ovarian Serous Cystadenocarcinoma TCGA, Pan Cancer Atlas, Ovarian Serous Cystadenocarcinoma TCGA, Nature 2011 and High-Grade Serous OC.

DISCUSSION

Ovarian cancer is a serious gynaecological disease affecting women wide world with highly mortality rate (Malvezzi *et al.*, 2016). Mouse models have been utilized to explore the molecular mechanism by which miRNAs activate or inhibit the development and metastasis of ovarian cancer which in turn can exhibit potential therapies (Dwi Sandhiutami *et al.*, 2019; Pei *et al.*, 2020). Recently, microarray technology with the aid of bioinformatic approaches have revealed their essential roles in the identification of novel genes involved in the oncogenesis, diagnosis and treatment of ovarian cancer (Xue *et al.*, 2021). In this study, we screened miRNA expression analysis using GEO microarray datasets to detect down-regulated miRNAs in the ovarian tissue from three previous studies (Dong *et al.*, 2019; Ji Nam *et al.*, 2016; Vuong *et al.*, 2017). Downregulated miRNAs were identified in cancer and normal ovarian samples. three of the downregulated-miRNAs, miR-378a-3p, -5p and miR-378c were common among GSE database. These miRNAs were determined to be significantly downregulated in cancerous samples that are in agreement with the previous results of these studies. These miRNAs functioned as tumour suppressors in the different types of cancer (Castellani *et al.*, 2022; Cui *et al.*, 2020; Pan *et al.*, 2019; Wang *et al.*, 2015; Yu *et al.*, 2021). Several miRNA target genes identified through PPI analysis play central roles in the cellular division, survival and death of tumour (Mohammadi *et*

al., 2022). In addition, subnetwork cluster analysis identified 13 hub module genes of downregulated miRNAs. Interestingly, the common target genes of miR-378a-3p, -5p and miR-378c were crucially enriched in molecular function GO term-linked to protein binding which is responsible for the activation of various signalling proteins that are potentially related to the regulation of proliferation and metastasis of ovarian cancer (Altomare *et al.,* 2004; Bileck *et al.,* 2022; Q. Guo *et al.,* 2021). KEGG analysis was predominately enriched in Ras signaling pathway. Ras proteins are activated by several external and internal factors in ovarian cancers such as mitogenic molecules, growth factors and hormones suggesting that the Ras signaling pathway controls an enormous amount of cellular activities including the regulation of cell cycle, proliferation, survival and apoptosis in response to these external and internal stimuli in ovarian carcinoma (Fan *et al.,* 2012; Liang *et al.,* 2019). Dysregulation of the Ras signaling pathway is implicated in different types of cancers including ovarian tumor (Li *et al.,* 2022). Among these common hub miRNA target genes, six of them revealed high genetic mutation and low cancer prognosis. FLT1 (fms-related tyrosine kinase 1.) along with other kinase genes were positively activated by the Ras signaling pathway which appeared to have a strong relationship with ovarian cancer prognosis (Stany *et al.,* 2011). Moreover, reports have documented that expression of the FLT1 was observed in the OC patient through induction of the tumour growth and this gene could be utilised as a biomarker of ovarian cancer (Eskander and Tewari, 2014; Sopo *et al.,* 2019).

The strength of the present in silico-analysis study was reporting the common downregulated expressed miRNAs as a pivotal prognostic biomarker in ovarian cancer. We used a broad search strategy of the NCBI-GEO database contained ovarian cancer and miRNAs. We performed the GEOR2 software analysis and found that miR-378a3p, 5p and miR-378c were common downregulated miRNAs among three databases GSE83693, GSE119055 and GSE98391. Our results revealed that the low levels of these miRNAs implied weak clinical outcomes in ovarian cancers. The major limitations of our study, Firstly, we only screened the downregulated expressed miRNAs in the tissue of ovarian cancer patients and healthy tissue. However, miRNA expression levels may alter in ovarian cancer patients with different tumour stages, sample types, ages, and health statuses. Secondly, validation of the identified potential biomarkers was not conducted in the in vivo models of ovarian cancer due to the limited sample sizes of these datasets. Future experiments are required to verify the defined potential biomarker and demonstrated the functional evaluation of these miRNA in regulating ovarian cancer invasion and metastasis, and to validate the involvement of miRNA target genes in the diagnosis and prognosis of ovarian cancer patients.

Conclusion

These results confirmed that the down-regulation of miR-378a-3p, -5p and miR-378c are notably connected with poor survival of OC patients. Functional annotation analysis suggested that the improved prognosis of ovarian cancer is associated with the expression and activation of miRNA target genes including FLT1 (fms-related tyrosine kinase 1.) which involved in the stimulation of Ras signalling pathway. The current findings may contribute to the development of a novel cancer therapeutical strategy by identifying the target genes involved in ovarian cancer.

Author Declaration: The authors of this study declare no conflict of interests.

Statement of Ethics Approval: The datasets were collected from previously published studies; therefore, ethical approval was not needed for this study.

Authors' Contributions Statement: BTM, SIM and BKA have participated in the conception and design of this study. Methods and data analysis were conducted by BTM. BTM drafted the manuscript and all authors read and approved the final manuscript**.**

REFERENCES

Alshamrani, A.A., 2020. Roles of microRNAs in Ovarian Cancer Tumorigenesis: Two Decades Later, What Have We Learned? *Frontiers in Oncology,* 10, 1084. https://doi.org/10.3389/fonc.2020.01084

Altomare, D.A., Wang, H.Q., Skele, K.L., Rienzo, A. De, Klein-Szanto, A.J., Godwin, A.K., Testa, J.R., 2004. AKT and mTOR phosphorylation is frequently detected in ovarian cancer and can be targeted to disrupt ovarian tumor cell growth. *Oncogene,* 23, 5853–5857. https://doi.org/10.1038/sj.onc.1207721

Bileck, A., Bortel, P., Kriz, M., Janker, L., Kiss, E., Gerner, C., Del Favero, G., 2022. Inward Outward Signaling in Ovarian Cancer: Morpho-Phospho-Proteomic Profiling Upon Application of Hypoxia and Shear Stress Characterizes the Adaptive Plasticity of OVCAR-3 and SKOV-3 Cells . *Frontiers in Oncology,* 11: 746411. doi: 10.3389/fonc. 2021. 746411.

Calura, E., Fruscio, R., Paracchini, L., Bignotti, E., Ravaggi, A., Martini, P., Sales, G., Beltrame, L., Clivio, L., Ceppi, L., Di Marino, M., Fuso Nerini, I., Zanotti, L., Cavalieri, D., Cattoretti, G., Perego, P., Milani, R., Katsaros, D., Tognon, G., Sartori, E., Pecorelli, S., Mangioni, C., D'Incalci, M., Romualdi, C., Marchini, S., 2013. miRNA Landscape in Stage I Epithelial Ovarian Cancer Defines the Histotype Specificities. *Clinical Cancer Research,* 19, 4114 LP – 4123. https://doi.org/10.1158/1078-0432.CCR-13-0360

Castellani, G., Buccarelli, M., Lulli, V., Ilari, R., De Luca, G., Pedini, F., Boe, A., Felli, N., Biffoni, M., Pilozzi, E., Marziali, G., Ricci-Vitiani, L., 2022. MiR-378a-3p Acts as a Tumor Suppressor in Colorectal Cancer Stem-Like Cells and Affects the Expression of MALAT1 and NEAT1 lncRNAs . *Frontiers in Oncology,* 12:867886. doi: 10.3389/fonc.2022.867886

Chen, S.-N., Chang, R., Lin, L.-T., Chern, C.-U., Tsai, H.-W., Wen, Z.-H., Li, Y.-H., Li, C.-J., Tsui, K.-H., 2019. MicroRNA in Ovarian Cancer: Biology, Pathogenesis, and Therapeutic Opportunities. *International Journal of Environmental Research and Public Health,* 16, 1510. https://doi.org/10.3390/ijerph16091510

Chin, C.-H., Chen, S.-H., Wu, H.-H., Ho, C.-W., Ko, M.-T., Lin, C.-Y., 2014. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Systems Biology,* 8, 1–7.

Choi, J.-H., Wong, A.S.T., Huang, H.-F., Leung, P.C.K., 2007. Gonadotropins and Ovarian Cancer. *Endocrine Reviews,* 28, 440–461. https://doi.org/10.1210/er.2006-0036

Cui, Z., Sun, S., Liu, Q., Zhou, X., Gao, S., Peng, P., Li, Q., 2020. MicroRNA-378-3p/5p suppresses the migration and invasiveness of oral squamous carcinoma cells by inhibiting KLK4 expression. *Biochemistry and Cell Biology,* 98, 154–163.

Davis, M., Rauh-Hain, J.A., Andrade, C., Boruta II, D.M., Schorge, J.O., Horowitz, N.S., May, T., Del Carmen, M.G., 2014. Comparison of clinical outcomes of patients with clear cell and endometrioid ovarian cancer associated with endometriosis to papillary serous carcinoma of the ovary. *Gynecologic Oncology,* 132, 760–766.

Desai, A., Xu, J., Aysola, K., Qin, Y., Okoli, C., Hariprasad, R., Chinemerem, U., Gates, C., Reddy, A., Danner, O., Franklin, G., Ngozi, A., Cantuaria, G., Singh, K., Grizzle, W., Landen, C., Partridge, E.E., Rice, V.M., Reddy, E.S.P., Rao, V.N., 2014. Epithelial ovarian cancer: An

overview. *World Journal of Translational Medicine,* 3, 1–8. https://doi.org/10.5528/wjtm.v3.i1.1

Dong, S., Wang, R., Wang, H., Ding, Q., Zhou, X., Wang, J., Zhang, K., Long, Y., Lu, S., Hong, T., 2019. HOXD-AS1 promotes the epithelial to mesenchymal transition of ovarian cancer cells by regulating miR-186-5p and PIK3R3. *Journal of Experimental & Clinical Cancer Research,* 38, 1–13.

Dwi Sandhiutami, N.M., Wuyung, P.E., Arozal, W., Louisa, M., Rahmat, D., 2019. Ovarian Cancer Animal Models for Preclinical Studies and Development of Ovarian Cancer Drugs. *Journal Ilmu Kefarmasian Indonesia,* Vol 17 No 2 JIFIDO - 10.35814/jifi.v17i2.734 .

Eskander, R.N., Tewari, K.S., 2014. Incorporation of anti-angiogenesis therapy in the management of advanced ovarian carcinoma Mechanistics, review of phase III randomized clinical trials, and regulatory implications. *Gynecologic Oncology,* 132, 496–505. https://doi.org/https://doi.org/10.1016/j.ygyno.2013.11.029

Fan, H.-Y., Liu, Z., Mullany, L.K., Richards, J.S., 2012. Consequences of RAS and MAPK activation in the ovary: The good, the bad and the ugly. *Molecular and Cellular Endocrinology,* 356, 74–79.https://doi.org/https://doi.org/10.1016/j.mce.2011.12.005

Gadducci, A., Cosio, S., 2020. Therapeutic Approach to Low-Grade Serous Ovarian Carcinoma: State of Art and Perspectives of Clinical Research. *Cancers (Basel),* 12, 1336. https://doi.org/10.3390/cancers12051336

Guo, Q., Wu, Y., Guo, X., Cao, L., Xu, F., Zhao, H., Zhu, J., Wen, H., Ju, X., Wu, X., 2021. The RNA-Binding Protein CELF2 Inhibits Ovarian Cancer Progression by Stabilizing FAM198B. *Molecular Therapy Nucleic Acids,* 23, 169–184. https://doi.org/https://doi.org/10.1016/j.omtn.2020.10.011

Guo, T., Dong, X., Xie, S., Zhang, Ling, Zeng, P., Zhang, Lin, 2021. Cellular Mechanism of Gene Mutations and Potential Therapeutic Targets in Ovarian Cancer. *Cancer Management and Research,* 13, 3081.

Ji Nam, E., Kim, S., Sang Lee, T., Jung Kim, H., Yun Lee, J., Wun Kim, S., Hoon Kim, J., Tae Kim, Y., 2016. Primary and recurrent ovarian high-grade serous carcinomas display similar microRNA expression patterns relative to those of normal ovarian tissue. *Oncotarget;* Vol 7, No 43.

Köbel, M., Kalloger, S.E., Huntsman, D.G., Santos, J.L., Swenerton, K.D., Seidman, J.D., Gilks, C.B., on behalf of the Cheryl Brown Ovarian Cancer Outcomes Unit of the British Columbia Cancer Agency, V.B.C., 2010. Differences in Tumor Type in Low-stage Versus High-stage Ovarian Carcinomas. *International Journal of Gynecological Pathology* ,29.

Li, M., Tang, Y., Zuo, X., Meng, S., Yi, P., 2022. Loss of Ras GTPase-activating protein SH3 domain-binding protein 1 (G3BP1) inhibits the progression of ovarian cancer in coordination with ubiquitin-specific protease 10 (USP10).*Bioengineered,* , 13, 721–734. https://doi.org/10.1080/21655979.2021.2012624

Liang, F., Ren, C., Wang, J., Wang, S., Yang, L., Han, X., Chen, Y., Tong, G., Yang, G., 2019. The crosstalk between STAT3 and p53/RAS signaling controls cancer cell metastasis and cisplatin resistance via the Slug/MAPK/PI3K/AKT-mediated regulation of EMT and autophagy. *Oncogenesis,* 8, 59. https://doi.org/10.1038/s41389-019-0165-8

Malvezzi, M., Carioli, G., Rodriguez, T.,

Negri, E., La Vecchia, C., 2016. Global trends and predictions in ovarian cancer mortality. *Annals of Oncology,* 27, 2017–2025. https://doi.org/10.1093/annonc/mdw306

Mohammadi, K., Mohammadpour, H., Jamshidian, F., 2022. Differential Expression of miR-589-5p, miR-569 and c-Fos gene in Oral Squamous Cell Carcinoma. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology,* 14, 1–12. https://doi.org/10.21608/eajbsc.2022.212827

Mohammed, B.T., Sontakke, S.D., Ioannidis, J., Duncan, W.C., Donadeu, F.X., 2017. The adequate corpus luteum: Mir-96 promotes luteal cell survival and progesterone production. *Journal of Clinical Endocrinology and Metabolism,* 102, 2188–2198. doi.org/10.1210/jc.2017-00259

Nam, E.J., Yoon, H., Kim, S.W., Kim, H., Kim, Y.T., Kim, J.H., Kim, J.W., Kim, S., 2008. MicroRNA expression profiles in serous ovarian carcinoma. *Clinical Cancer Research,* 14, 2690–2695.

Pan, X., Zhao, L., Quan, J., Liu, K., Lai, Y., Li, Z., Zhang, Z., Xu, J., Xu, W., Guan, X., 2019. MiR-378a-5p acts as a tumor suppressor in renal cell carcinoma and is associated with the good prognosis of patients. *American Journal of Translational Research,* 11, 2207.

Pei, Y., Li, K., Lou, X., Wu, Y., Dong, X., Wang, W., Li, N., Zhang, D., Cui, W., Pei, Y., Li, K., Lou, X., Wu, Y., Dong, X., Wang, W., Li, N., Zhang, D., Cui, W., 2020. miR-1299/NOTCH3/TUG1 feedback loop contributes to the malignant proliferation of ovarian cancer Corrigendum in /10.3892/or. 2023. 8533. *Oncology Reports,* 44, 438–448. https://doi. org/10.3892/or. 2020.7623

Ricciardi, E., Baert, T., Ataseven, B., Heitz, F., Prader, S., Bommert, M., Schneider, S., du Bois, A., Harter, P., 2018. Low-grade serous ovarian carcinoma. *Geburtshilfe Frauenheilkd,* 78, 972–976. https://doi.org/10.1055/a-0717-5411

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research,* 13, 2498–2504.

Sherman, B.T., Huang, D.W., Tan, Q., Guo, Y., Bour, S., Liu, D., Stephens, R., Baseler, M.W., Lane, H.C., Lempicki, R.A., 2007. DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. *BMC Bioinformatics,* 8, 426. https://doi.org/10.1186/1471-2105-8-426

Sontakke, S.D., Mohammed, B.T., McNeilly, A.S., Donadeu, F.X., 2014. Characterization of microRNAs differentially expressed during bovine follicle development. *Reproduction,* 148, 271–283. https://doi.org/10.1530/REP-14-0140

Sopo, M., Anttila, M., Hämäläinen, K., Kivelä, A., Ylä-Herttuala, S., Kosma, V.-M., Keski-Nisula, L., Sallinen, H., 2019. Expression profiles of VEGF-A, VEGF-D and VEGFR1 are higher in distant metastases than in matched primary high grade epithelial ovarian cancer. *BMC Cancer,* 19, 584. https://doi.org/10.1186/s12885-019-5757-3

Stany, M.P., Vathipadiekal, V., Ozbun, L., Stone, R.L., Mok, S.C., Xue, H., Kagami, T., Wang, Y., McAlpine, J.N., Bowtell, D., Gout, P.W., Miller, D.M., Gilks, C.B., Huntsman, D.G., Ellard, S.L., Wang, Y.-Z., Vivas-Mejia, P., Lopez-Berestein, G., Sood, A.K., Birrer, M.J., 2011. Identification of Novel

Therapeutic Targets in Microdissected Clear Cell Ovarian Cancers. *PLoS One,* 6, e21121.

Sticht, C., De La Torre, C., Parveen, A., Gretz, N., 2018. miRWalk: An online resource for prediction of microRNA binding sites. *PLoS One,* 13, e0206239.

Vilming Elgaaen, B., Olstad, O.K., Haug, K.B.F., Brusletto, B., Sandvik, L., Staff, A.C., Gautvik, K.M., Davidson, B., 2014. Global miRNA expression analysis of serous and clear cell ovarian carcinomas identifies differentially expressed miRNAs including miR-200c-3p as a prognostic marker. *BMC Cancer,* 14, 80. https://doi.org/10.1186/1471-2407-14-80

Vuong, N.H., Salah Salah, O., Vanderhyden, B.C., 2017. 17β-Estradiol sensitizes ovarian surface epithelium to transformation by suppressing Disabled-2 expression. *Scientific Reports,* 7, 16702. https://doi.org/10.1038/s41598-017-16219-2

Wang, Z., Ma, B., Ji, X., Deng, Y., Zhang, T., Zhang, X., Gao, H., Sun, H., Wu, H., Chen, X., Zhao, R., 2015. MicroRNA-378-5p suppresses cell proliferation and induces apoptosis in colorectal cancer cells by targeting BRAF. *Cancer Cell International,* 15, 40. https://doi.org/10.1186/s12935-015-0192-2

Xue, B., Li, S., Jin, X., Liu, L., 2021. Bioinformatics analysis of mRNA and miRNA microarray to identify the key miRNA-mRNA pairs in cisplatin-resistant ovarian cancer. *BMC Cancer,* 21, 452. https://doi.org/10.1186/s12885-021-08166-z

Yoshikawa, N., Kajiyama, H., Mizuno, M., Shibata, K., Kawai, M., Nagasaka, T., Kikkawa, F., 2014. Clinicopathologic features of epithelial ovarian carcinoma in younger vs. older patients: analysis in Japanese women. *Journal of Gynecologic Oncology,* 25, 118–123.

Yu, Q., Zheng, B., Ji, X., Li, P., Guo, Z., 2021. miR-378c suppresses Wilms tumor development via negatively regulating CAMKK2. *Acta Biochimica et Biophysica Sinica. (Shanghai),* 53, 739–747.

Zhang, S., Zhang, X., Fu, X., Li, W., Xing, S., Yang, Y., 2018. Identification of common differentially-expressed miRNAs in ovarian cancer cells and their exosomes compared with normal ovarian surface epithelial cell cells. *Oncology Letters,* 16, 2391–2401. https://doi.org/10.3892/ ol. 2018.8954

Supplementary Tables

Table 1 Common down-regulated miRNAs from 3 ovarian cancer database

Expression	**GSE83693 vs GSE119055**	**GSE119055 vs GSE98391**	**GSE83693 GSE119055 GSE98391**
Downregulated miRNAs	miR-130b-3p miR-200b-3p miR-200a-5p miR-185-5p miR-183-5p miR-141-3p miR-21-3p miR-224-5p miR-200c-3p miR-200b-5p miR-18b-5p miR-18a-5p miR-21-5p miR-425-5p miR-3200-3p miR-200a-3p miR-182-5p miR-93-5p miR-885-5p	miR-378c miR-378a-3p	miR-378a-5p

Table 2. Common target genes of the three downregulated miRNAs from 3 ovarian cancer database

Downregulated miRNAs	Target genes
miR-378a-3p, miR-378a-5p, miR-378c	SAMD4A,, ZHX3,BTG2,NHLRC3,LDB3,KCNK6,VWA5A,CORO2A,FBXO31, RASA4B, SYT2, PPM1A, CDC42BPA, CIITA, ZNF70, CMTM3, AGPS, DCUN1 D5, LONRF2, DUSP18, ARMC6, MAD2L1, RBPMS, TFEC, RIC3, DCX, OAS1, P AQR3, PAWR, FBXL20, LAMTOR3, RAB15, JRK, XIAP, MKLN1, FOXN2, HPC AL4, HMBOX1, ELL, AGBL1, SLC30A2, RALB, CBLN2, RBMXL1,\TBC1D16, PABPN1, ITGA10, STK24, BMF, BATF2, HTR4, SHE, USP15, NTRK2, SAMHD1 , ARL17B, KCNK9, TESPA1, HM13, RPRD1A, C5AR2, CPEB4, TAL1, ERBIN, SL C5A10, MBTPS2, PEDS1, ESYT2, SLAMF7, PGR, PHEX, E2F2, PDE4DIP, CCDC 157, TAB3, GUCD1, GSG1, C10orf105, TMEM135, UBE2V1, FHIP1A, CYB5A, C X3CR 1, XKR4, FBXL4, ALG1, SETD4, CSNK2A1, AAK1, RMND1, IL1R1, USP 8, RBM48, STARD7, ACTR3, ITIH5, RNF41, TSPAN3, ABL2, MECP2, ABCG1, COG6, SMAD3, SLC16A7, OR56A1, YTHDF3, KATNAL1, CFAP61, SPRYD4,R IOX2, STMN3, CALHM4, PRRG4, VPS36, ORMDL3, RNF144A, CRHR2, ATP2C 1, RCC2, MAPK9, IREB2, MTF1, ASPH, SV2B, DYNLL2, KCNJ9, DBT, ITGB6, G RIN2B, TRIM26, ARSB, N6AMT1, PAK3, CDC42, TUB, SCAMP5, RCAN3, CEN PC, SLC6A20, UBE2B, LRP10, MIEF1, MCL1, PTPN7, PTAR1, PSTPIP2, MVB1 2B, LYPD6, XPR1, AGO1, CCDC149, WDR31, ZBTB37, B3GNT3, CNR2, CDYL 2, UBE2R2, KIF6, ZNF766, SPOCK2, OPRD1, NCBP2, NAV1, RETREG3,PLAGL2 DCP2, MRPL49, PPM1L, MAP2, EDEM1, SPRY3, FBXW11, RPL13, CYREN, AZ IN2, ZNF517, INSR, GAS7, AMOTL1, PAICS, PPP2R2B, RRM2B, TMX2, RNF130 , CARF, BSND, TCF7, DTWD1, ADAM22, HIP1, RAET1E, RBP1, NF2, ITPRIP, TN R, WHRN, STUM, NPFFR1, ATG14, C11orf54, SAR1B, ELMOD1, WASF2, RNLS , GRAP2, RNF38, ZNF785, MRPL10, USP47, ZNF852, A1CF, MRPL52, FITM2, LO C401040, GJB1, RBFOX1, SLC25A35, ABHD2, CELF1, TSPYL4, EFHC1, PPFIA 3, ZNF24, PIKAP1, BACE2, LIF, NKAIN3, ABI2, AFAP1L1, KIF2A, PRRG1, HOX D13, NR2C2ABCC11, MGST1, FRYL, TTC21B, GNAO1, ZNF891, GANAB, KC NG4, MCFD2, DNMT3A, C1GALT1, ZNF740, ARMH4, GNG2, NTPCR, ALG11, UBE2L3, RUNDC1, MAVS, KNOP1, SDHAF1, TSPAN14, IGFBP5, MYOCD, C HMP4C, CCR7, NME9, KLHDC8A, FAM111A, FLT1, ZNF365, CCDC125, MPZ, TSC1, RCHY1, MOGAT3, ZNF268, POLR2F, TTC39A, POLDIP2, PABIR3, CCP G1, METTL2A, CEMIP, HLADPB1, AMMECR1L, HMG20A, PHACTR2, EHD4, ZNF589, CD70, SH2D1B, SGCD, CLSPN, CERT1, ECT2L, ELP2, ZNF207, MPRI P, LRRC57, LRAT, FBXL13, ERC1, EFCAB11, RUBCNL, ZNF652, DERL3, GP R180, LYRM2, CDH13, METTL8, NSD2, FARSB, SYT2, VAMP1, TAF7L, TFCP 2L1, SLC8A3, FAM86B1, TLR6, COPZ1, GPATCH2L, PAPPA, SMAGP, TOM1 L2, UBL4A, STEAP4, KSR1, NME6, IFT22, MRPS33, FHL2, PSMB2, SUGT1, AP1M1, CCDC134, KCNC3, ZNF124, GNG4, DRAM1, GALM, AMDHD2, RNF1 15, SPECC1, TNFAIP8L1, NXF1, HPGD, AK2, OSBPL6, NTRK3, NIPAL2, SLC7 A8, LAMC1, PDE1A, NFAM1, GPR26, MCTS1, IKBIP, DLG2,\PML,BRAP,PPP 1R12B, PPARA, PIAS2, NFASC, SLC5A12, EPB41L2, BCL2L2PABPN1, FGF7, KREMEN1, ZDHHC9, SRSF10, LEPROTL1, ENSA, SRSF1, RAB29, BFAR, RLN 3, ETS1, HYKK, KLHDC10, SPATS1, RAB30, SDHC, GNB5, PTAFR, OXNAD1, PCDHB1, LRRFIP2, GXYLT1, DYRK1A, NCMAP, ZNHIT3, SCN8A, PEX19, CNP, DLGAP2, SPATA13, TTPAL, CCDC198, TMEM199, LGALS8, SYNPO, CEP44, DCTN5, RPS14, PRKCA, HIPK1, KLRD1, TP63, SEC22A,ERCC2.