Research Paper

Identification of the miRNA signature associated with survival in patients with ovarian cancer

Srinivasulu Yerukala Sathipati[1,2,3], Shinn-Ying Ho[2,4,5,6]

[1]Center for Precision Medicine Research, Marshfield Clinic Research Institute, Marshfield, WI 54449, USA
[2]Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsinchu, Taiwan
[3]Institute of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan
[4]Institute of Bioinformatics and Systems Biology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan
[5]Department of Biological Science and Technology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan
[6]Center For Intelligent Drug Systems and Smart Bio-Devices (IDS^{2}B), National Yang Ming Chiao Tung University, Hsinchu, Taiwan

Correspondence to: Shinn-Ying Ho; **email:** syho@mail.nctu.edu.tw
Keywords: miRNA signature, survival estimation, ovarian cancer, machine learning
Received: October 30, 2020 **Accepted:** March 23, 2021 **Published:** April 27, 2021

ABSTRACT

Ovarian cancer is a major gynaecological malignant tumor associated with a high mortality rate. Identifying survival-related variants may improve treatment and survival in patients with ovarian cancer. In this work, we proposed a support vector regression (SVR)-based method called OV-SURV, which is incorporated with an inheritable bi-objective combinatorial genetic algorithm for feature selection to identify a miRNA signature associated with survival in patients with ovarian cancer. There were 209 patients with miRNA expression profiles and survival information of ovarian cancer retrieved from The Cancer Genome Atlas database. OV-SURV achieved a mean correlation coefficient of 0.77±0.01and a mean absolute error of 0.69±0.02 years using 10-fold cross-validation. Analysis of the top ranked miRNAs revealed that the miRNAs, hsa-let-7f, hsa-miR-1237, hsa-miR-98, hsa-miR-933, and hsa-miR-889, were significantly associated with the survival in patients with ovarian cancer. Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed that four of these miRNAs, hsa-miR-182, hsa-miR-34a, hsa-miR-342, and hsa-miR-1304, were highly enriched in fatty acid biosynthesis, and the five miRNAs, hsa-let-7f, hsa-miR-34a, hsa-miR-342, hsa-miR-1304, and hsa-miR-24, were highly enriched in fatty acid metabolism. The prediction model with the identified miRNA signature consisting of prognostic biomarkers can benefit therapeutic decision making of ovarian cancer.

INTRODUCTION

Ovarian cancer is one of the deadliest types of gynaecological malignant tumors, resulting approximately 125,000 deaths worldwide each year [1]. Among ovarian cancer types, approximately 80–90% of cases are epithelial ovarian cancer. Some of the genetic risk factors for ovarian cancer include mutations in the tumor suppressor genes *BRCA1* and *BRCA2* [2]. Among patients with ovarian cancer, 8% have *BRCA1* mutation, 6% have *BRCA2* mutations, 6% have somatic *BRCA1/2* mutations, and 10% have *BRCA1* promoter inactivation [3]. Epithelial ovarian cancers spread to the adjacent organs first and later spreads to the liver and lungs, although bone and brain metastases are rarely observed. The survival time of patients with ovarian cancer varies at different stages; the 10-year survival rate is ~30% in all patients, and a recent study showed that after initial chemotherapy, the 5-year mortality rate was 65% [1, 4]. The treatment for ovarian cancer is determined by a combination of surgery and chemotherapy, either primary debulking surgery followed by chemotherapy or neo-adjuvant chemotherapy followed by interval debulking and chemotherapy [5]. Because of difficulties associated

with early-stage detection and mass screening, ovarian cancer is the most lethal among all gynaecologic cancers.

MicroRNAs (miRNAs) are small noncoding RNAs that have attracted increasing attention owing to their potential involvement in initiation, progression, and metastasis [6] of various cancers. Several studies have shown that the expression of noncoding RNAs is associated with various types of cancer; hence, these RNAs have been used for predicting cancer types [7–10]. Moreover, miRNA and gene expression profiling have been used for prediction of survival and as effective molecular diagnostic markers in various cancers [2, 11, 12]. Recently, relationship between miRNA profiles and ovarian tumors has been studied extensively [13]; this study revealed that most miRNAs are downregulated in epithelial ovarian cancer and thus are associated with genomic copy number loss [14], indicating that miRNA expression is affected by genomic alterations. Studies on the dysregulation of miRNA expression in cancer may help to target oncogenes and to suppress their functions, thereby improving cancer treatment.

There are many studies that have discovered miRNA signatures for the survival analysis and prediction of progression in ovarian cancer. For instance, Shih et al. identified two miRNAs miR-410 and miR-645 that are negatively associated with the overall survival in advanced serous ovarian carcinoma [15]. Bagnoli et al. identified 35 miRNAs to create a prognostic model, and predicted risk of progression or relapse in ovarian cancer [16]. Besides the ovarian cancer, miRNA signatures have been used to predict survival in other cancer types, such as glioblastoma [17], lung cancer [18], and hepatocellular carcinoma [19]. Additionally, miRNA signatures have used to predict new subtypes of cancers. For instance, mRNA and miRNA expression signatures were used to predict subtype of serous ovarian cancer [20]. Bhattacharyya et al. used miRNA signatures for identifying subtypes of breast cancer [21]. Recently, various effective computational methods have been developed to understand the miRNA disease association [22, 23]. Hence, miRNA signatures are proven to be important factors for predicting survival and subtypes in cancers.

Various machine learning methods have been developed for cancer typing and diagnosis, including subtyping of liver cancer [24], breast cancer [25], lung cancer [26], and ovarian cancer [27]. These studies have used different machine learning models, such as support vector machines (SVMs) and artificial neural networks, along with various validation methods. Notably, SVMs have already been used to categorise the stages of ovarian cancer. De Smet et al. classified 31 patients with ovarian cancer into a stage I subgroup and an advanced-stage subgroup using least-square SVMs and principle component analysis, showing good accuracy [28]. Hartmann et al. used the cDNA gene expression profiles of 79 patients with ovarian carcinoma to predict early and late relapse [29]. Gevaert et al. have also estimated advanced-stage cisplatin resistance and stage I cisplatin sensitivity by means of microarray data obtained from 20 patients with ovarian cancer and observed a poor prediction [30]. Lisowska et al. utilised SVMs and Kaplan-Meier curves to predict disease-free survival and overall survival among 97 patients with ovarian cancer and observed no statistical difference in gene expression in the validation sets [31].

Only a few studies have therefore addressed prediction of cancer survival by means of a machine learning approach. One of the general issues with the use of machine learning methods is data quality, which is affected by noise and by missing and repetitive data. Thus, adequate data processing protocols are necessary for successful use of machine learning for the above purpose; feature selection plays a key role in reducing the number of unessential features to obtain a robust model. Overfitting is another general issue with the use of machine learning methods for predictive analysis of cancer. One of the known ways to avoid overfitting is cross-validation [32]. During cross-validation, a training dataset is used as a model and for testing. A comparison of model selection methods suggested that the 10-fold cross-validation (10-CV) method is the best approach for model selection [33].

Currently established treatments often fail to cure ovarian cancer. Substantial efforts have been made to find better therapeutic modalities to cure this cancer. As described above, to identify survival-related variants in ovarian cancer, miRNA expression data are often examined. Accordingly, the main aim of this work was to identify the miRNA signature that could estimate the survival of patients with ovarian cancer. In this work, we used The Cancer Genome Atlas (TCGA) database to retrieve miRNA expression profiles from 209 patients with ovarian cancer. We proposed a support vector regression (SVR)-based estimator (OV-SURV) for identification of miRNA signatures for the prediction of survival time in patients with ovarian cancer. As far as authors concern, this is the first study to use miRNA expression profiles and SVR to estimate the survival time in patients with ovarian cancer. OV-SURV identified a miRNA signature that associated with survival time of patients with ovarian cancer. We conducted 10-CV to assess the performance of OV-SURV. As a result, OV-SURV achieved a mean correlation coefficient of 0.77±0.01, with a mean absolute error of 0.69±0.02 years when comparing real and estimated survival time. Further, we seek to characterize the miRNA signature, as well as to

understand the molecular mechanism in ovarian cancer survival by using bioinformatics approaches.

OV-SURV approach was based on an SVR and included an optimal feature selection method referred to as the inheritable bi-objective combinatorial genetic algorithm (IBCGA) [34]. Previously, an optimised SVR method was used to estimate the survival time of patients with glioblastoma [17], lung adenocarcinoma [18] and neuroblastoma [35]. This study could be ascribed to the following factors. Firstly, identifying the survival associated biomarkers in cancers are always necessary which could contribute to the prevention and diagnosis of cancers. Secondly, we customized the optimization method and tuned the parameters of OV-SURV, and prioritized the miRNAs of the signature to explore their roles in ovarian cancer. Thirdly, the prognostic power of the top ranked miRNAs in ovarian cancer was validated using survival curves. Fourthly, the biological significance of the identified miRNA signature was analyzed in terms of pathway analysis and functional annotations. Finally, importance of the top ranked miRNAs in ovarian cancer was verified by experimental literature. Further, we performed differential expression analysis and gene target prediction for the top ranked miRNAs. We hope that our findings can help identify new ovarian cancer-related miRNAs and contribute to the development of prognostic biomarkers in near future.

RESULTS AND DISCUSSION

Identification of miRNA signature associated with survival time

First, we attempted to estimate the survival time of patients with ovarian cancer using miRNA expression profiles. We used a dataset containing 209 patients with ovarian cancer along with the expression profiles of 415 miRNA and survival time data. OV-SURV included the optimal feature selection algorithm IBCGA and identified a miRNA signature consisting of 39 miRNAs that could be used to predict the survival time of patients with ovarian cancer.

The OV-SURV-average yielded a correlation coefficient of 0.77±0.01 and mean absolute error of 0.69±0.02 years between real and estimated survival time. The data strongly suggested that the identified miRNAs were effective at estimating the survival time of patients with ovarian cancer. The system flow chart of this work is depicted in Figure 1.

We compared this OV-SURV method with standard multiple linear regression [36], LASSO [37], and Elastic net [38]. The LASSO method resulted in a correlation coefficient and a mean absolute error of 0.48 and 1.00 years, respectively. The Elastic net method resulted in a correlation coefficient and a mean absolute error of 0.55 and 1.00 years, respectively. The multiple linear regression method resulted in a correlation coefficient and a mean absolute error of 0.66 and 0.84 years, respectively. A comparison of these results is shown in Table 1. The correlation plots between the estimated and real survival time obtained using OV-SURV, LASSO, Elastic net, and multiple linear regression are shown in Figure 2.

Further, OV-SURV was validated on an independent dataset from the TCGA database to evaluate the prediction performance. The dataset consisting of 160 living patients who suffer from ovarian cancer with follow-up time was used for the validation. The follow-up times of 160 patients were up to 5 years and the mean and standard deviation of the follow-up times were 20.42 ± 16.30 months. The mean estimated survival time of OV-SURV was 33.85 ± 5.24 months. There were 123 patients whose estimated survival time was longer than their actual follow-up time. The result revealed that OV-SURV achieved the classification accuracy of 77% for estimating the survival of patients with ovarian cancer. The mean absolute error of predicting the remaining 37 patients was 0.93 years which is slightly longer than the 0.69 years in Table 1. The prediction performance of OV-SURV on an independent test cohort was measured based on our previous study [18]. The prediction result of OV-SURV for each of the 160 patients is shown in Supplementary Figure 1.

Ranking of the identified miRNA signature

To prioritize the miRNAs of the identified miRNA signature, miRNAs were ranked based on their contributions to the survival estimation using main effect difference (MED) analysis. There are 39 miRNAs in the identified miRNA signature. The 39 miRNAs of the signature, miRNA IDs, and MED scores are shown in Table 2. The top 10 miRNAs ranked by MED analysis were hsa-miR-19b, hsa-let-7f, hsa-miR-323, hsa-miR-1978, hsa-miR-128, hsa-miR-1237, hsa-miR-486, hsa-miR-98, hsa-miR-933, and hsa-miR-889, and these miRNAs were then analyzed further. Among the miRNAs in the signature identified by OV-SURV, these 10 miRNAs were significantly associated with survival time. We then assessed the involvement of these miRNAs and their corresponding genes in cancer and various biological pathways.

Validating the prognostic potential of top 10 ranked miRNAs

We validated the prognostic power of the top 10 ranked miRNAs using Kaplan-Meier (KM) survival curves. The

TCGA dataset contains 486 tumor samples were considered for this analysis. Survival analysis was performed using Kaplan-Meir plotter [39]. Five of the top 10 ranked miRNAs, hsa-let-7f, hsa-miR-1237, hsa-miR-98, hsa-miR-933, and hsa-miR-889, were significantly associated with survival in patients with ovarian cancer. The survival groups were distributed using the log-rank test, a p-value<0.05 was considered the cut-off to describe the statistical significance in the survival analysis. These five miRNAs, hsa-let-7f, hsa-miR-1237, hsa-miR-98, hsa-miR-933, and hsa-miR-889, have p-values of 0.01, 0.00033, 0.007, 4.9e-0.5, and 0.011, respectively; and hazard ratios of 0.73, 0.61, 0.73, 1.62, and 1.4, respectively.

Pathway analysis of the identified miRNA signature

The biological relevance of the selected miRNAs was analyzed using the DIANA tool. The pathway analysis helps to determine whether these miRNAs were statistically significant in Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways, including signalling pathways, cell processes and cancer pathways. The analysis revealed that the top 10 selected miRNAs were

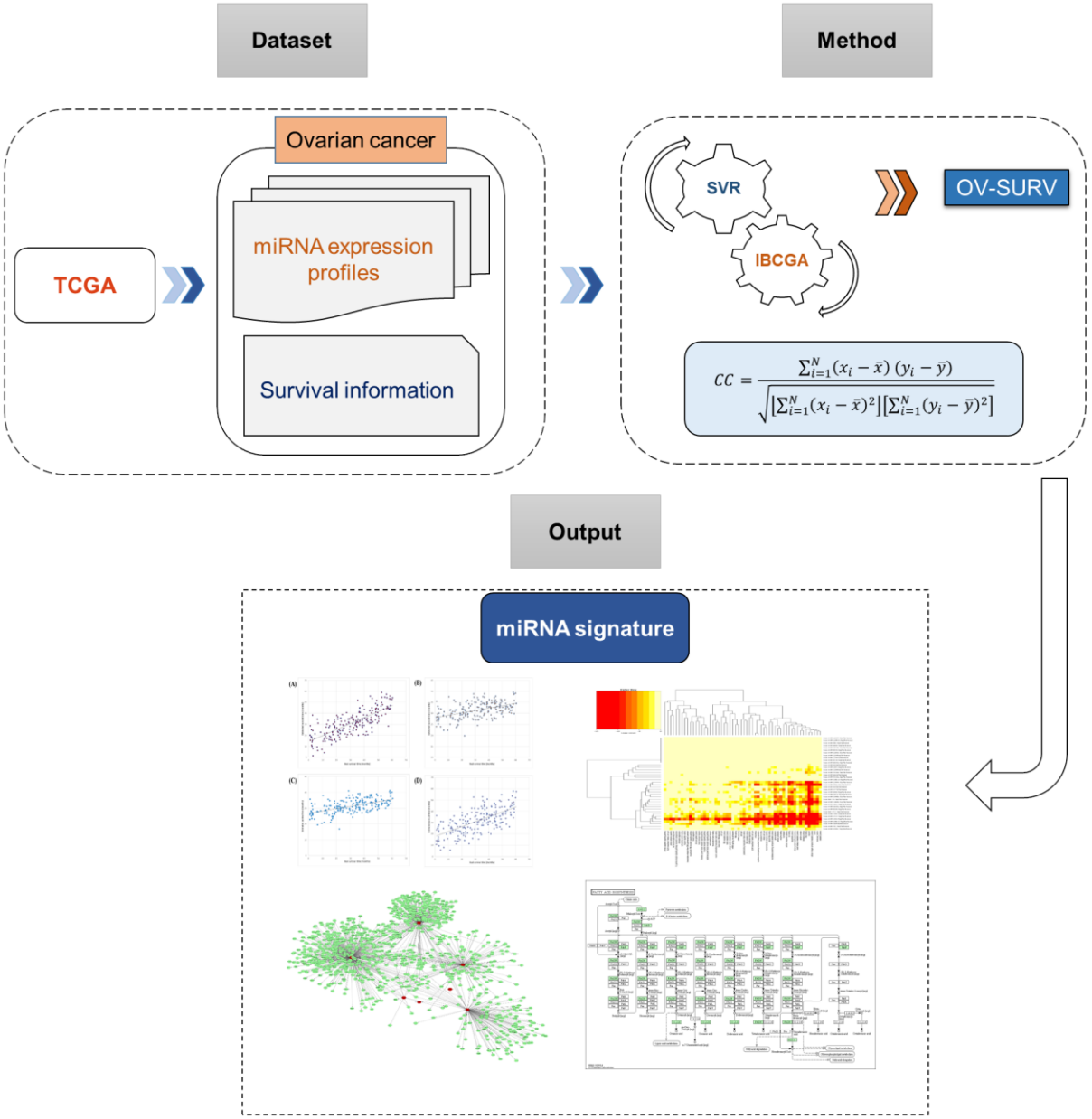


Figure 1. System flowchart of the current study describing the discovery of miRNA signature for predicting survival time in ovarian cancer.

Table 1. Prediction of the performance of OV-SURV.

Method	Identified miRNAs	10-CV correlation coefficient	Mean absolute error (years)
LASSO	18	0.48	1.00
Elastic net	32	0.55	1.00
Multiple linear regression	18	0.66	0.84
OV-SURV	39	0.76	0.69
OV-SURV-mean	34.63	0.77±0.01	0.69±0.02

involved in several KEGG pathways, the most significant pathways including the transforming growth factor-β signalling pathway (hsa04350; p=3.29e-06), proteoglycans in cancer (hsa05205; p=7.93e-05), sphingolipid metabolism (hsa00600; p=0.0001), hippo signalling pathway (hsa04390; p=0.0005), and adherens junction (hsa04520; p=0.009). The details of the KEGG pathway analysis for the top 10 ranked miRNAs and their number of target genes are shown in Supplementary Table 1.

Additionally, the KEGG analysis of all 39 miRNAs in the signature revealed that few miRNAs were highly enriched in fatty acid biosynthesis, fatty acid metabolism, ECM/receptor interactions, and the hippo signalling pathway. The most highly significant pathway found in the KEGG pathway analysis was fatty acid biosynthesis (p<1E-325). There were four miRNAs (hsa-miR-182, hsa-miR-34a, hsa-miR-342, and hsa-miR-1304) that were highly enriched in fatty acid biosynthesis (hsa00061) and targeted genes such as *FASN*, *ACSL4*, and *ACACA*. Additionally, five miRNAs, namely, hsa-let-7f, hsa-miR-34a, hsa-miR-342, hsa-miR-1304, and hsa-miR-24, targeted the genes *FASN*, *PTPLB*, *SCD*, *ACSL4*, *ECHS1*, and *TECR*, which were all involved in fatty acid metabolism (p=1.02E-14). Fatty acid synthase (FAS) is an enzyme responsible for the synthesis of fatty acids and has been identified in most human carcinomas. FAS

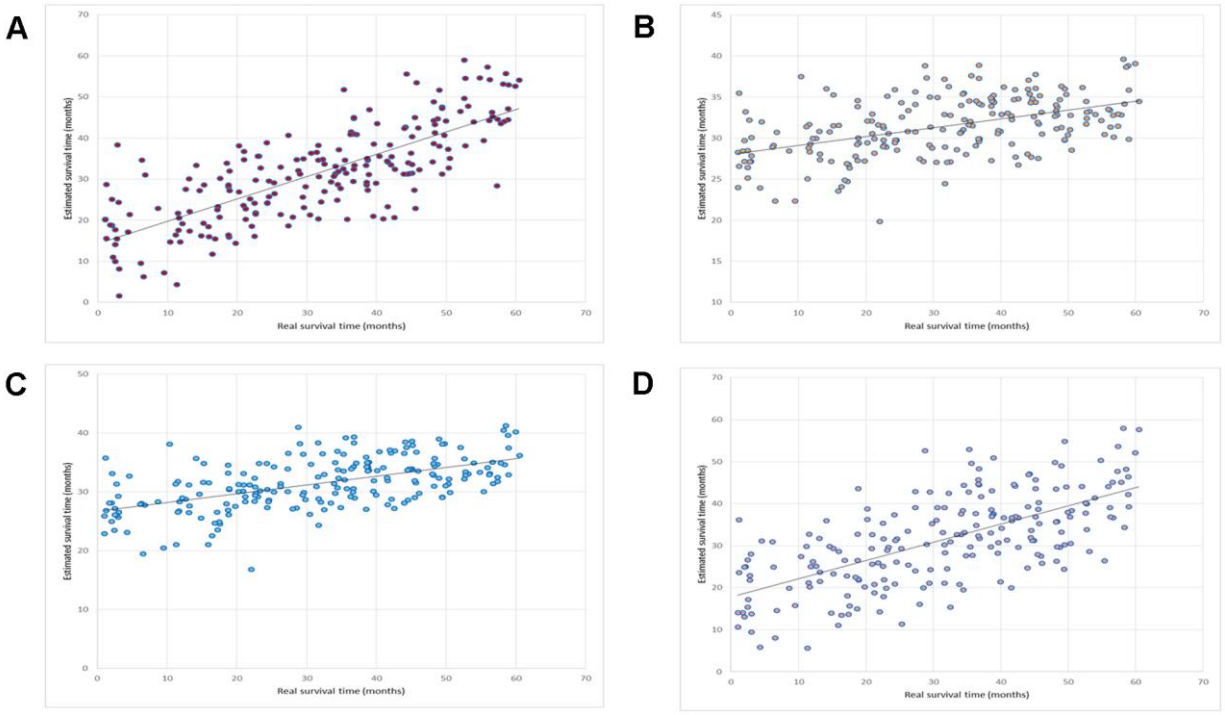


Figure 2. Prediction of the performance of OV-SURV. (**A**) OV-SURV achieved a correlation coefficient of 0.76. (**B**) LASSO yielded a correlation coefficient of 0.48. Real survival time in months is shown on the X-axis, and estimated survival time in months is shown on the Y-axis. (**C**) Elastic net obtained a correlation coefficient of 0.55. (**D**) Multiple linear regression obtained a correlation coefficient of 0.66. Real survival time in months is shown on the X-axis, and estimated survival time in months is shown on the Y-axis.

Table 2. Main effect difference (MED) scores of miRNA signatures.

Rank	MiRNA	MED
1	hsa-miR-19b	0.986621
2	hsa-let-7f	0.978907
3	hsa-miR-323	0.952427
4	hsa-miR-1978	0.867624
5	hsa-miR-128	0.821022
6	hsa-miR-1237	0.812224
7	hsa-miR-486	0.706714
8	hsa-miR-98	0.607252
9	hsa-miR-933	0.585211
10	hsa-miR-889	0.574037
11	hsa-miR-301b	0.568445
12	hsa-miR-514	0.561426
13	hsa-miR-935	0.530403
14	hsa-miR-653	0.494192
15	hsa-miR-1251	0.49296
16	hsa-miR-616	0.469875
17	hsa-miR-662	0.455425
18	hsa-miR-182	0.455374
19	hsa-miR-1245	0.435942
20	hsa-miR-200c	0.432309
21	hsa-miR-34a	0.36374
22	hsa-miR-187	0.353238
23	hsa-miR-190	0.343974
24	hsa-miR-342	0.329363
25	hsa-miR-513a	0.314319
26	hsa-miR-146b	0.306926
27	hsa-miR-1197	0.277773
28	hsa-miR-577	0.265914
29	hsa-miR-185	0.232879
30	hsa-miR-212	0.179705
31	hsa-miR-874	0.127656
32	hsa-miR-1304	0.122577
33	hsa-miR-106b	0.093269
34	hsa-miR-31	0.080119
35	hsa-miR-320d	0.04015
36	hsa-miR-1295	0.037462
37	hsa-miR-664	0.032954
38	hsa-let-7b	0.013824
39	hsa-miR-24	0.000766

protein expression is associated with poor prognosis in both prostate and breast cancers [40, 41]. An *in vivo* microarray study revealed that there was a significant relationship between phosphorylated AKT and the expression of FAS; inhibition of FAS activity resulted in down regulation of phosphorylated AKT, which initiates apoptosis in ovarian cancer cells [42]. Inhibition of FAS and activation of AMP-activated protein kinase is selectively cytotoxic to SKOV3 human ovarian cancer cells [43]. Additionally, inhibition of FAS is significantly associated with survival in xenograft models [44]. This analysis suggested that the selected miRNAs were

relevant to cancer and related biological signalling pathways. The heatmap showing KEGG pathway enrichment analysis of the miRNA signature is shown in Figure 3A.

Thus, this analysis indicated that fatty acid synthesis in ovarian cancer cells played an essential role in ovarian cancer progression. The identified miRNAs involved in fatty acid synthesis and fatty acid metabolism may

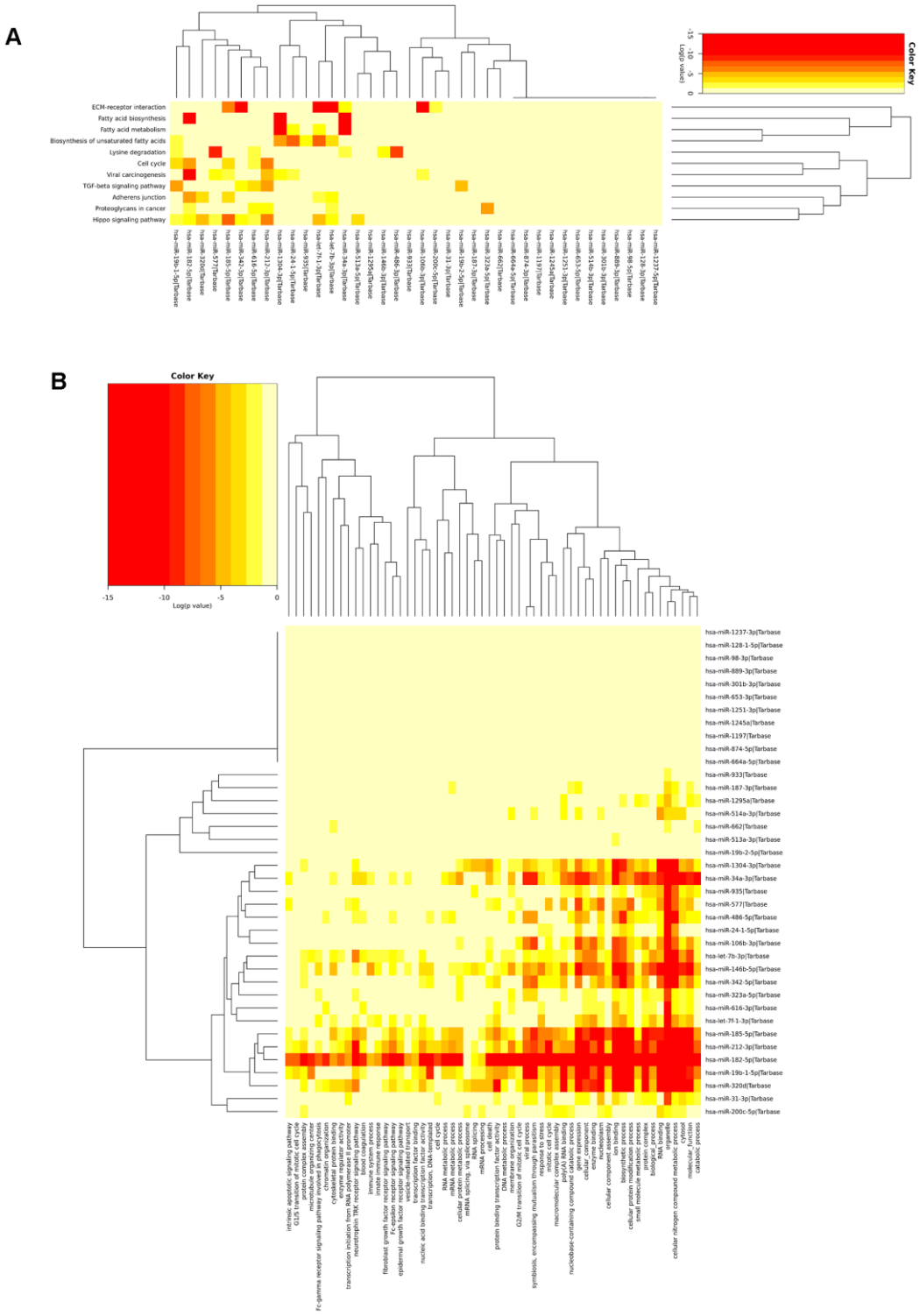


Figure 3. Biological significance of the miRNA signature. (**A**) miRNAs highly enriched in fatty acid metabolism, fatty acid biosynthesis, ECR receptor, and lysine degradation and (**B**) The miRNAs were found to be involved in different cellular, molecular, and biological pathways.

possess important roles in ovarian cancer survival. The results of the KEGG pathway analysis for the miRNA signature and its target genes (various pathways, numbers of miRNAs involved, and the corresponding p-values) are shown in Supplementary Table 2. Fatty acid biosynthesis and fatty acid metabolism flow diagrams are shown in Supplementary Figures 2, 3.

Functional annotations of the miRNA signature

Functional annotations of the identified miRNA signature were analyzed using GO enrichment analysis. GO annotation analysis revealed that the top 10 ranked miRNAs were involved in several biological processes (BPs), cellular component (CCs), and molecular functions (MFs). The top 10 ranked miRNAs were involved in several BPs, such as gene expression (GO:0010467; p<1e-325), biosynthetic process (GO:0009058; p<1e-325), cellular nitrogen compound metabolic process (GO:0044403; p<1e-325), symbiosis, encompassing mutualism through parasitism (GO:0044403; p=2.78e-14), and viral process (GO:0016032; p=3.14e-13) by targeting 82, 360, 419, 68, and 62 genes, respectively, to name a few.

The top 10 ranked miRNAs were involved in various CCs, includes organelle (GO:0043226; p<1e-325), cytosol (GO:0005829; p=4.46e-13), protein complex (GO:0043234; p=2.55e-11), nucleoplasm (GO:0005654; p=9.93e-11), and microtubule organizing center (GO:0005815; p=0.004). The top 10 ranked miRNs involved in MFs, such as ion binding (GO:0043167; p<1e-325), RNA binding (GO:0003723; p=2.22e-15), enzyme binding (GO:0019899; p=2.09e-08), poly(A) RNA binding (GO:0044822; p=1.16e-07), protein binding transcription factor activity (GO:0000988; p=4.34e-05), and nucleic acid binding transcription factor activity (GO:0001071;p=0.006). In GO annotation analysis, among the BPs most genes involved in cellular nitrogen compound metabolic process (419 genes), in CCs most genes involved in organelle (834 genes), and in MFs, most genes are involved in ion binding (447 genes). The details of top 10 ranked miRNAs involvement in GO annotations is listed in Supplementary Table 3.

Go enrichment analysis revealed that miRNA signature is highly enriched in gene expression, catabolic process, RNA binding, cytosol, cellular nitrogen compound metabolic process, viral process cellular protein modification process, organelles, and enzyme binding. GO analysis of the miRNA signature is shown in Figure 3B. The results of the GO analysis for the miRNA signature and its target genes are shown in Supplementary Table 4.

Expression difference of the top ranked miRNAs across stages

Further, we wished to enquire about the relative expression levels of the top ranked miRNAs across different stages of ovarian cancer. We employed relative miRNA expression analysis across stages of ovarian cancer using UALCAN web portal [45]. The expression analysis results provide the significant expression difference of top 10 ranked miRNAs across different stages of patients with ovarian cancer. Of the top 10 ranked miRNAs, except the miRNA, hsa-miR-1978, remaining miRNAs are significantly expressed across stage 2, 3, and 4 of patients with ovarian cancer. Statistical significance of the relative miRNA expression across stages of ovarian cancer patients is provided in Supplementary Table 5. Box-whisker plot representation of relative expression difference across stages of ovarian cancer patients is given for the top ranked miRNAs in Supplementary Figure 4.

Roles of the top ranked miRNAs in ovarian cancer

The roles of the top 10 ranked miRNAs in ovarian cancer were analyzed using experimentally validated literature.

hsa-miR-19b: A quantitative real-time PCR study involving ovarian cancer cells showed that hsa-miR-19b was significantly expressed between cancer and control group with a p-value of 0.035 [46]. Over expression of hsa-miR-19b facilitated the invasion and migration of ovarian cancer cells (CAOV-3) and serves as an oncogenic role in ovarian cancer progression [47].

hsa-let-7f: Zheng et al. identified lower expression levels of hsa-let-7f in ovarian cancer samples when compared to controls, further lower levels of hsa-let-7f associated with poor prognosis of patients with ovarian cancer [48]. Elevated expression level of let-7f was found in primary ovarian carcinomas and suggesting a role of this miRNA in tumor progression [49].

hsa-miR-323: *In silico* miRNA expression analysis in ovarian cancer reported that dysregulation of hsa-miR-323 in ovarian cancer cells [50]. Differential expression of miR-323 was observed in epithelial ovarian cancer cells and miR-323 was significantly down-regulated in epithelial ovarian cancer cell lines compared to ovarian surface epithelium cells [14].

hsa-miR-128: hsa-miR-128 downregulates the colony stimulating factor-1, a key regulator of ovarian cancer, resulted into reduction of cell motility and adhesion in ovarian cancer cells [51].

hsa-miR-486: hsa-miR-486 downregulated the estrogen receptor alpha (ERα)-mediated olfactomedin 4, which contributes to ovarian cancer progression in cancer cells [52].

hsa-miR-98: A qRT-PCR study epithelial ovarian cancer revealed that hsa-miR-98 regulates the cisplatin resistance of epithelial ovarian cancer cells and was associated with poor outcome of patients with ovarian cancer [53].

A summary of the top 10 ranked miRNAs and their roles in ovarian cancer are shown in Table 3. In summary, six miRNAs, namely, hsa-miR-19b, hsa-let-7f, hsa-miR-323, hsa-miR-128, hsa-miR-486, and hsa-miR-98, had experimentally validated support to confirm their associations in ovarian cancer. The remaining four miRNAs, such as hsa-miR-1978, hsa-miR-1237, hsa-miR-933, and hsa-miR-889, were not previously reported to have roles in ovarian cancer. However, they were actively involved in other types of cancer, such as kidney cancer [54], breast cancer [55], gastric cancer [56], and squamous cell carcinoma [57]. Additionally, we verified the importance of these four miRNAs, hsa-miR-1978, hsa-miR-1237, hsa-miR-933, and hsa-miR-889 in ovarian cancer by performing overall survival analysis using Kaplan-Meir survival curves. The miRNAs, hsa-miR-1978, hsa-miR-1237, hsa-miR-933, and hsa-miR-889 obtained p-values of 0.22, 0.01, <0.001, and <001, respectively. The three miRNAs, hsa-miR-1237, hsa-miR-933, and hsa-miR-889 were significantly associated with the overall survival in patients with ovarian cancer. The KM survival plots for hsa-miR-889, hsa-miR-1237, and hsa-miR-933 are shown in Supplementary Figure 5.

Furthermore, we validated the involvement of top 10 ranked miRNAs in various cancers including ovarian cancer using experimentally validated studies. All of the top 10 ranked miRNAs were involved in major types of cancers, in which 6 miRNAs, hsa-miR-19b, hsa-let-7f, hsa-miR-323, hsa-miR-128, hsa-miR-486, and hsa-miR-98 were involved in ovarian cancer progression. The top 10 ranked miRNAs and experimentally validated evidences across various cancers is listed in Supplementary Table 6.

Differential expression of miRNA signature in ovarian cancer

Furthermore, to examine the involvement of the miRNA signature and the expression levels of the miRNAs of the signature in ovarian cancer, we predicted the association of the miRNA signature with ovarian cancer using dbDEMC 2.0 and HMDD v3.0. The prediction results showed that 29 miRNAs were differentially expressed in ovarian cancer. The expression levels of the remaining 10 miRNAs had not been previously reported in ovarian cancer, although these miRNAs were known to be actively involved in other major cancer types. These results suggested that these 10 miRNAs, including hsa-miR-323, hsa-miR-1978, hsa-miR-933, hsa-miR-616, hsa-miR-1245, hsa-miR-19b-2, hsa-miR-342, hsa-miR-513a, hsa-miR-577, and hsa-miR-1295, should be further evaluated to determine their roles in ovarian cancer survival. The prediction list of the identified miRNA signature associated with ovarian cancer is shown in Supplementary Table 7.

Additionally, we predicted the miRNA gene target network for the top ranked miRNAs using Cytoscape version 3.6. There are total 5009 predicted gene targets, in which 3893 interactions are predicted using MicroCosm version 5, and 116 interactions are predicted using TargetScan version 6.2. The miRNA-gene target interaction network is visualized in Supplementary Figure 6, for better visualization, only gene targets predicted by TargetScan are shown.

CONCLUSIONS

Considering experimental limitations and rapidly growing biological datasets, machine learning models are becoming necessary for the identification of cancer-related miRNAs, e.g., in cancer diagnosis. MiRNA profiling is now extensively used in cancer research and cancer treatment. Recently, various miRNAs have been shown to play important roles in ovarian cancer invasion and metastasis. Some miRNAs were significantly expressed in cancer tissues when compared to the normal tissues. For instance, miR-199a, and miR-200 were significantly expressed in ovarian cancer tissues when compared to the normal tissues [58]. There are some miRNAs which have been identified as prognostic biomarkers in ovarian cancer. For example, miR-1183, miR-126, miR-802, and miR-139 showed significant association with prognosis [59]. Bagnoli et al. identified miRNAs to predict early relapse/progression of ovarian cancer patients [16]. However, there are few studies using machine learning with optimization techniques to estimate the survival time of patients with ovarian cancer. Hence, in this work, we proposed a survival estimation method, called OV-SURV, that identified an miRNA signature associated with survival in patients with ovarian cancer. Machine learning methods for analysis of cancer survival are often faced the problem of overfitting. Accordingly, we addressed this issue in our OV-SURV model, which contained an optimal feature selection algorithm. OV-SURV yielded promising results and selected an informative miRNA signature that was associated with survival in patients with ovarian cancer. MED ranking revealed the 10 miRNAs that were the most significant with respect to

Table 3. Summary of top 10 ranked miRNAs involved in ovarian cancer.

Rank	miRNA	Cancer	Regulation	Source
1	hsa-miR-19b	ovarian cancer	up	[66]
2	hsa-let-7f	ovarian cancer	down	[49]
3	hsa-miR-323	ovarian cancer	down	[14]
4	hsa-miR-1978	-	-	-
5	hsa-miR-128	ovarian cancer	down	[51]
6	hsa-miR-1237	-	-	-
7	hsa-miR-486	ovarian cancer	down	[52]
8	hsa-miR-98	ovarian cancer	down	[53]
9	hsa-miR-933	-	-	-
10	hsa-miR-889	-	-	-

cancer survival. Validation of top ranked miRNAs using KM survival analysis revealed the prognostic power of the miRNAs, of top 10 ranked miRNAs, five miRNAs were significantly associated with survival in patients with ovarian cancer. Moreover, among the identified miRNA signature, the finding of the four miRNAs hsa-miR-486, hsa-miR-514, hsa-miR-200c, and hsa-miR-513a was consistent to those in the study [16] for the association with the prognosis in patients with ovarian cancer. The identified miRNAs have prognostic value in ovarian cancer.

The biological significance of these miRNAs was analyzed by KEGG pathway and GO annotations, which indicated that the selected miRNAs were significantly involved in the regulation of fatty acid biosynthesis, fatty acid metabolism, and several other relevant signalling and cancer pathways. Identified miRNAs are significantly expressed across different stages of ovarian cancer. These expression differences of miRNAs might also affect the survival in patients with ovarian cancer. Further, the importance of top ranked miRNAs in ovarian cancer is discussed. Six of the top 10 ranked miRNAs role in ovarian cancer was verified by experimental validated literature. This analysis revealed that remaining four miRNAs of the top 10 ranked miRNAs, such as hsa-miR-1978, hsa-miR-1237, hsa-miR-933, and hsa-miR-889, were not previously reported to have roles in ovarian cancer. However, their contribution to the survival estimation revealed that these are the important subjects to explore further in ovarian cancer. Differential expression analysis of miRNA signature shown 29 of 39 miRNAs of the signature were significantly expressed in ovarian cancer cells. The miRNA-target network was constructed to visualize the target interactions of the top ranked miRNAs, derived from interaction databases.

The above findings showed the ability of the OV-SURV method to identify miRNA signatures that could predict survival in patients with ovarian cancer. Although the prediction performance of OV-SURV is promising, this method may be further improved by increasing the data size. Nonetheless, our model performed well compared with other machine learning methods in terms of 10-CV. The identified miRNAs were proven here to be relevant to ovarian cancer and other cancers. Because of increasing cancer-related mortality, computational predictive methods are necessary for the rapid identification of potential biomarkers. We believe that the miRNAs identified here will help to develop new prognostic or diagnostic biomarkers for ovarian cancer.

MATERIALS AND METHODS

Dataset

We obtained miRNA expression data from patients with ovarian cancer using the TCGA database. The number of patients with ovarian cancer in the TCGA database was 586. We downloaded level-3 miRNA expression data from the TCGA portal, and the miRNA profiling was performed using the Illumina HiSeq 2000 miRNA sequencing platform. We filtered the dataset using the following criteria: (i) only patients with clinical data and survival information were included; (ii) to statistically remove the bias, the patients with a survival period of less than 30 days were excluded; and (iii) all patient lists and the corresponding survival periods were merged into a single data file to eliminate duplicate entries. After this filtering process, we identified 209 patients with the expression profiles of 415 miRNAs along with corresponding clinical data and days to death. Additionally, we used the dataset consisting of 160 living patients who suffer from ovarian cancer with follow-up time for the validation. Clinical information of the independent test cohort is listed in Supplementary Table 8.

OV-SURV

The proposed method OV-SURV was designed to estimate the survival time of patients with ovarian cancer by identifying a miRNA signature and then to discover their potential roles of miRNA biomarkers in ovarian cancer. SVM is effective for solving classification and regression problems [60] and can interpret the property values of a large number of samples in a multidimensional space. Owing to its effective regression abilities, SVR has been used to solve many biological prediction problems. The optimisation problem of ν-SVR for finding a model w can be defined as follows:

$$\min [\{\frac{1}{2} w^T (\varnothing(x_i) + b) + C(\nu\varepsilon + \frac{1}{m}\sum_{i=1}^{m}(\xi_i + \xi_i^*))\}] \quad (1)$$

Here, $0 \le \nu \le 1$, $\xi_i \ge 0$, $\xi_i^* \ge 0$, $(\boldsymbol{x}_1, y_1)\ldots(\boldsymbol{x}_m, y_m) \in R^d \times R$ are training data, y_i is the target values, $\boldsymbol{x}_i$ is the feature vector. We used d is the number of features in each instance and m is the number of training instance where d=415 and m=209 in this study. Let C>0 be the regularisation parameter, ε≥0 be an error sensitivity parameter, and b is a constant. The parameters C and ε are used to avoid overfitting the training data. The parameters C, ε, a Gaussian kernel γ and the feature selection are simultaneously optimized by OV-SURV while maximizing the estimation accuracy.

IBCGA

The feature selection algorithm IBCGA uses an intelligent evolutionary algorithm (IEA) [34], which can efficiently solve large parameter optimisation problems, and is good at deriving an optimised SVR model with feature selection. IBCGA can automatically select a small set of informative features from a large number of candidate features. OV-SURV identifies a signature of informative miRNAs associated with ovarian cancer survival based on the cooperation of IBCGA and ν-SVR using the objective function of maximising the correlation coefficient (CC) in terms of 10-CV.

$$CC = \frac{\sum_{i=1}^{N}(x_i - \bar{x})\,(z_i - \bar{z})}{\sqrt{\left[\sum_{i=1}^{N}(x_i - \bar{x})^2\right]\left[\sum_{i=1}^{N}(z_i - \bar{z})^2\right]}} \quad (2)$$

where x_i and z_i are real and predicted survival time of the i-th miRNA, $\bar{x}$ and $\bar{z}$ are the corresponding means, and N is the total number of instances. Mean absolute error (MAE) is also used for estimating the prediction performance. The MAE is defined as follows

$$MAE = \frac{1}{N}\sum_{i=1}^{N}\left|x - z\right|^2 \quad (3)$$

In this work, the LibSVM package [61] was used for implementation of ν-SVR.

The chromosome of IEA comprises 415 binary genes representing miRNAs for feature selection and three 4-bit genes for encoding parameters γ, C, and ν of ν-SVR. The chromosome encoding was designed as described in previous studies [17, 34]. The IBCGA can simultaneously obtain a set of solutions, Xr, where $r = r_{end}, r_{end+}1, \ldots, r_{start}$ in a single run. The feature selection algorithm IBCGA used can be described as follows:

Step 1: (Initialisation) Randomly generate an initial population of $Npop$ individuals. In this work, $Npop$=50, r_{start} = 50, r_{end} = 10, $r = r_{start}$.

Step 2: (Evaluation) Evaluate the fitness value of all individuals using the fitness function (CC) of 10-CV.

Step 3: (Selection) Use a conventional method of tournament selection that selects a winner from two randomly selected individuals to generate a mating pool.

Step 4: (Crossover) Select two parents from the mating pool to perform an orthogonal array crossover operation of IEA.

Step 5: (Mutation) Apply a conventional bit mutation operator to parameter genes and a swap mutation to the binary genes for keeping r selected features. The best individual was not mutated for the elite strategy.

Step 6: (Termination test) If the stopping condition for obtaining the solution Xr is satisfied, output the best individual as the solution Xr. Otherwise, go to Step 2.

Step 7: (Inheritance) If $r > r_{end,}$ randomly change one bit in the binary genes for each individual from 1 to 0; decrease the number r by one and go to Step 2. Otherwise, stop the algorithm.

Step 8: (Output) Obtain a set of m miRNAs from the chromosome of the best solution Xm among the solutions Xr, where $r = r_{end}, r_{end+}1, \ldots, r_{start}$.

Multiple linear regression analysis

We employed multiple regression to estimate the survival time in patients with ovarian cancer [36]. A general multiple linear regression can be defined as

$$y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n + \varepsilon \quad (4)$$

KEGG pathway and GO term analysis

We used the DIANA-mirPath version 3 web-based server to analyze the miRNA profiles [62]. The DIANA-micro-T-CDS algorithm provided the predicted miRNA targets for the pathway analysis. The p-value threshold was set to 0.05 and Fishers's exact test (hypergeometric distribution) was used for the enrichment analysis. We utilised the Network of Cancer Genes (NCG) [63], miRTarBase [64], and GeneCards [65] databases to obtain the cancer-related genes and predict gene targets.

Availability of data and materials

All the data used in this analysis can be found at TCGA data portal [https://cancergenome.nih.gov/].

AUTHOR CONTRIBUTIONS

Srinivasulu Yerukala Sathipati and Shinn-Ying Ho designed the system, participated in manuscript preparation, and carried out the detailed study. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

This work was supported by Ministry of Science and Technology ROC under the contract numbers MOST 109-2221-E-009-129-, 109-2740-B-400-002-, 108-2221-E-009-127-, and 108-2218-E-029-004-, and was financially supported by the "Center For Intelligent Drug Systems and Smart Bio-devices (IDS^{2}B)" from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Barnholtz-Sloan JS, Schwartz AG, Qureshi F, Jacques S, Malone J, Munkarah AR. Ovarian cancer: changes in patterns at diagnosis and relative survival over the last three decades. Am J Obstet Gynecol. 2003; 189:1120–27. https://doi.org/10.1067/S0002-9378(03)00579-9 PMID:14586365

2. Welsh JB, Zarrinkar PP, Sapinoso LM, Kern SG, Behling CA, Monk BJ, Lockhart DJ, Burger RA, Hampton GM. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. Proc Natl Acad Sci USA. 2001; 98:1176–81. https://doi.org/10.1073/pnas.98.3.1176 PMID:11158614

3. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011; 474:609–15. https://doi.org/10.1038/nature10166 PMID:21720365

4. Quinn MJ, d'Onofrio A, Møller B, Black R, Martinez-Garcia C, Møller H, Rahu M, Robertson C, Schouten LJ, La Vecchia C, Boyle P. Cancer mortality trends in the EU and acceding countries up to 2015. Ann Oncol. 2003; 14:1148–52. https://doi.org/10.1093/annonc/mdg307 PMID:12853360

5. Morgan RJ Jr, Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Behbakht K, Chen LM, Copeland L, Crispens MA, DeRosa M, Dorigo O, Gershenson DM, Gray HJ, Hakam A, et al. Ovarian Cancer, Version 1.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2016; 14:1134–63. https://doi.org/10.6004/jnccn.2016.0122 PMID:27587625

6. Munding JB, Adai AT, Maghnouj A, Urbanik A, Zöllner H, Liffers ST, Chromik AM, Uhl W, Szafranska-Schwarzbach AE, Tannapfel A, Hahn SA. Global microRNA expression profiling of microdissected tissues identifies miR-135b as a novel biomarker for pancreatic ductal adenocarcinoma. Int J Cancer. 2012; 131:E86–95. https://doi.org/10.1002/ijc.26466 PMID:21953293

7. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Ménard S, Palazzo JP, Rosenberg A, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005; 65:7065–70. https://doi.org/10.1158/0008-5472.CAN-05-1783 PMID:16103053

8. Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S, Calin GA, Volinia S, Liu CG, Scarpa A, Croce CM. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J Clin Oncol. 2006; 24:4677–84. https://doi.org/10.1200/JCO.2005.05.5194 PMID:16966691

9. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh S, Cheng CL, Yu CJ, Lee YC, Chen HS, Su TJ, Chiang CC, Li HN, et al. MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell. 2008; 13:48–57.

https://doi.org/10.1016/j.ccr.2007.12.008
PMID:18167339

10. Kim S, Park T, Kon M. Cancer survival classification using integrated data sets and intermediate information. Artif Intell Med. 2014; 62:23–31. https://doi.org/10.1016/j.artmed.2014.06.003 PMID:24997860

11. Santarelli L, Gaetani S, Monaco F, Bracci M, Valentino M, Amati M, Rubini C, Sabbatini A, Pasquini E, Zanotta N, Comar M, Neuzil J, Tomasetti M, Bovenzi M. Four-miRNA Signature to Identify Asbestos-Related Lung Malignancies. Cancer Epidemiol Biomarkers Prev. 2019; 28:119–26. https://doi.org/10.1158/1055-9965.EPI-18-0453 PMID:30257964

12. Shen Y, Ding Y, Ma Q, Zhao L, Guo X, Shao Y, Niu C, He Y, Zhang F, Zheng D, Wei W, Liu F. Identification of Novel Circulating miRNA Biomarkers for the Diagnosis of Esophageal Squamous Cell Carcinoma and Squamous Dysplasia. Cancer Epidemiol Biomarkers Prev. 2019; 28:1212–20. https://doi.org/10.1158/1055-9965.EPI-18-1199 PMID:30988139

13. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008; 110:13–21. https://doi.org/10.1016/j.ygyno.2008.04.033 PMID:18589210

14. Zhang L, Volinia S, Bonome T, Calin GA, Greshock J, Yang N, Liu CG, Giannakakis A, Alexiou P, Hasegawa K, Johnstone CN, Megraw MS, Adams S, et al. Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc Natl Acad Sci USA. 2008; 105:7004–09. https://doi.org/10.1073/pnas.0801615105 PMID:18458333

15. Shih KK, Qin LX, Tanner EJ, Zhou Q, Bisogna M, Dao F, Olvera N, Viale A, Barakat RR, Levine DA. A microRNA survival signature (MiSS) for advanced ovarian cancer. Gynecol Oncol. 2011; 121:444–50. https://doi.org/10.1016/j.ygyno.2011.01.025 PMID:21354599

16. Bagnoli M, Canevari S, Califano D, Losito S, Maio MD, Raspagliesi F, Carcangiu ML, Toffoli G, Cecchin E, Sorio R, Canzonieri V, Russo D, Scognamiglio G, et al, and Multicentre Italian Trials in Ovarian cancer (MITO) translational group. Development and validation of a microRNA-based signature (MiROvaR) to predict early relapse or progression of epithelial ovarian cancer: a cohort study. Lancet Oncol. 2016; 17:1137–46. https://doi.org/10.1016/S1470-2045(16)30108-5 PMID:27402147

17. Yerukala Sathipati S, Huang HL, Ho SY. Estimating survival time of patients with glioblastoma multiforme and characterization of the identified microRNA signatures. BMC Genomics. 2016 (Suppl 13); 17:1022. https://doi.org/10.1186/s12864-016-3321-y PMID:28155650

18. Yerukala Sathipati S, Ho SY. Identifying the miRNA signature associated with survival time in patients with lung adenocarcinoma using miRNA expression profiles. Sci Rep. 2017; 7:7507. https://doi.org/10.1038/s41598-017-07739-y PMID:28790336

19. Zhang J, Chong CC, Chen GG, Lai PB. A Seven-microRNA Expression Signature Predicts Survival in Hepatocellular Carcinoma. PLoS One. 2015; 10:e0128628. https://doi.org/10.1371/journal.pone.0128628 PMID:26046780

20. Bentink S, Haibe-Kains B, Risch T, Fan JB, Hirsch MS, Holton K, Rubio R, April C, Chen J, Wickham-Garcia E, Liu J, Culhane A, Drapkin R, et al. Angiogenic mRNA and microRNA gene expression signature predicts a novel subtype of serous ovarian cancer. PLoS One. 2012; 7:e30269. https://doi.org/10.1371/journal.pone.0030269 PMID:22348002

21. Bhattacharyya M, Nath J, Bandyopadhyay S. MicroRNA signatures highlight new breast cancer subtypes. Gene. 2015; 556:192–98. https://doi.org/10.1016/j.gene.2014.11.053 PMID:25485717

22. Jin S, Zeng X, Fang J, Lin J, Chan SY, Erzurum SC, Cheng F. A network-based approach to uncover microRNA-mediated disease comorbidities and potential pathobiological implications. NPJ Syst Biol Appl. 2019; 5:41. https://doi.org/10.1038/s41540-019-0115-2 PMID:31754458

23. Chen X, Sun LG, Zhao Y. NCMCMDA: miRNA-disease association prediction through neighborhood constraint matrix completion. Brief Bioinform. 2021; 22:485–96. https://doi.org/10.1093/bib/bbz159 PMID:31927572

24. Yerukala Sathipati S, Ho SY. Novel miRNA signature for predicting the stage of hepatocellular carcinoma. Sci Rep. 2020; 10:14452. https://doi.org/10.1038/s41598-020-71324-z PMID:32879391

25. Yerukala Sathipati S, Ho SY. Identifying a miRNA signature for predicting the stage of breast cancer. Sci Rep. 2018; 8:16138.

https://doi.org/10.1038/s41598-018-34604-3
PMID:30382159

26. Gevaert O, De Smet F, Timmerman D, Moreau Y, De Moor B. Predicting the prognosis of breast cancer by integrating clinical and microarray data with Bayesian networks. Bioinformatics. 2006; 22:e184–90. https://doi.org/10.1093/bioinformatics/btl230 PMID:16873470

27. Tseng CJ, Lu CJ, Chang CC, Chen GD, Cheewakriangkrai C. Integration of data mining classification techniques and ensemble learning to identify risk factors and diagnose ovarian cancer recurrence. Artif Intell Med. 2017; 78:47–54. https://doi.org/10.1016/j.artmed.2017.06.003 PMID:28764872

28. De Smet F, Pochet NL, Engelen K, Van Gorp T, Van Hummelen P, Marchal K, Amant F, Timmerman D, De Moor BL, Vergote IB. Predicting the clinical behavior of ovarian cancer from gene expression profiles. Int J Gynecol Cancer. 2006 (Suppl 1); 16:147–51. https://doi.org/10.1111/j.1525-1438.2006.00321.x PMID:16515583

29. Hartmann LC, Lu KH, Linette GP, Cliby WA, Kalli KR, Gershenson D, Bast RC, Stec J, Iartchouk N, Smith DI, Ross JS, Hoersch S, Shridhar V, et al. Gene expression profiles predict early relapse in ovarian cancer after platinum-paclitaxel chemotherapy. Clin Cancer Res. 2005; 11:2149–55. https://doi.org/10.1158/1078-0432.CCR-04-1673 PMID:15788660

30. Gevaert O, De Smet F, Van Gorp T, Pochet N, Engelen K, Amant F, De Moor B, Timmerman D, Vergote I. Expression profiling to predict the clinical behaviour of ovarian cancer fails independent evaluation. BMC Cancer. 2008; 8:18. https://doi.org/10.1186/1471-2407-8-18 PMID:18211668

31. Lisowska KM, Olbryt M, Dudaladava V, Pamuła-Piłat J, Kujawa K, Grzybowska E, Jarząb M, Student S, Rzepecka IK, Jarząb B, Kupryjańczyk J. Gene expression analysis in ovarian cancer - faults and hints from DNA microarray study. Front Oncol. 2014; 4:6. https://doi.org/10.3389/fonc.2014.00006 PMID:24478986

32. Stone M. Cross-Validatory Choice and Assessment of Statistical Predictions. Journal of the Royal Statistical Society Series B (Methodological). 1974; 36:111–47. https://doi.org/10.1111/j.2517-6161.1974.tb00994.x

33. Kohavi R. A study of cross-validation and bootstrap for accuracy estimation and model selection, in Proceedings of the 14th international joint conference on Artificial intelligence. Morgan Kaufmann Publishers Inc. 1995; 2:1137–43.

34. Ho SY, Chen JH, Huang MH. Inheritable genetic algorithm for biobjective 0/1 combinatorial optimization problems and its applications. IEEE Trans Syst Man Cybern B Cybern. 2004; 34:609–20. https://doi.org/10.1109/tsmcb.2003.817090 PMID:15369097

35. Yerukala Sathipati S, Sahu D, Huang HC, Lin Y, Ho SY. Identification and characterization of the lncRNA signature associated with overall survival in patients with neuroblastoma. Sci Rep. 2019; 9:5125. https://doi.org/10.1038/s41598-019-41553-y PMID:30914706

36. Aiken LS, West SG, Pitts SC. Pitts, Multiple Linear Regression, in Handbook of Psychology. 2003, John Wiley & Sons, Inc. https://doi.org/10.1002/0471264385.wei0219

37. Tibshirani R. Regression shrinkage and selection via the lasso: a retrospective. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 2011; 73:273–82. https://doi.org/10.1111/j.1467-9868.2011.00771.x

38. Zou H, Hastie T. Regularization and variable selection via the elastic net. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 2005; 67:301–20. https://doi.org/10.1111/j.1467-9868.2005.00503.x

39. Nagy Á, Lánczky A, Menyhárt O, Győrffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. Sci Rep. 2018; 8:9227. https://doi.org/10.1038/s41598-018-27521-y PMID:29907753

40. Swinnen JV, Roskams T, Joniau S, Van Poppel H, Oyen R, Baert L, Heyns W, Verhoeven G. Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. Int J Cancer. 2002; 98:19–22. https://doi.org/10.1002/ijc.10127 PMID:11857379

41. Milgraum LZ, Witters LA, Pasternack GR, Kuhajda FP. Enzymes of the fatty acid synthesis pathway are highly expressed in *in situ* breast carcinoma. Clin Cancer Res. 1997; 3:2115–20. PMID:9815604

42. Wang HQ, Altomare DA, Skele KL, Poulikakos PI, Kuhajda FP, Di Cristofano A, Testa JR. Positive feedback regulation between AKT activation and fatty acid synthase expression in ovarian carcinoma cells. Oncogene. 2005; 24:3574–82. https://doi.org/10.1038/sj.onc.1208463 PMID:15806173

43. Zhou W, Han WF, Landree LE, Thupari JN, Pinn ML, Bililign T, Kim EK, Vadlamudi A, Medghalchi SM, El Meskini R, Ronnett GV, Townsend CA, Kuhajda FP. Fatty acid synthase inhibition activates AMP-activated protein kinase in SKOV3 human ovarian cancer cells. Cancer Res. 2007; 67:2964–71. https://doi.org/10.1158/0008-5472.CAN-06-3439 PMID:17409402

44. Pizer ES, Wood FD, Heine HS, Romantsev FE, Pasternack GR, Kuhajda FP. Inhibition of fatty acid synthesis delays disease progression in a xenograft model of ovarian cancer. Cancer Res. 1996; 56:1189–93. PMID:8640795

45. Chandrashekar DS, Bashel B, Balasubramanya SA, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BV, Varambally S. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017; 19:649–58. https://doi.org/10.1016/j.neo.2017.05.002 PMID:28732212

46. Penyige A, Márton É, Soltész B, Szilágyi-Bónizs M, Póka R, Lukács J, Széles L, Nagy B. Circulating miRNA Profiling in Plasma Samples of Ovarian Cancer Patients. Int J Mol Sci. 2019; 20:4533. https://doi.org/10.3390/ijms20184533 PMID:31540229

47. Liu DT, Yao HR, Li YY, Song YY, Su MY. MicroRNA-19b promotes the migration and invasion of ovarian cancer cells by inhibiting the PTEN/AKT signaling pathway. Oncol Lett. 2018; 16:559–65. https://doi.org/10.3892/ol.2018.8695 PMID:29963131

48. Zheng H, Zhang L, Zhao Y, Yang D, Song F, Wen Y, Hao Q, Hu Z, Zhang W, Chen K. Plasma miRNAs as diagnostic and prognostic biomarkers for ovarian cancer. PLoS One. 2013; 8:e77853. https://doi.org/10.1371/journal.pone.0077853 PMID:24223734

49. Vaksman O, Stavnes HT, Kaern J, Trope CG, Davidson B, Reich R. miRNA profiling along tumour progression in ovarian carcinoma. J Cell Mol Med. 2011; 15:1593–602. https://doi.org/10.1111/j.1582-4934.2010.01148.x PMID:20716115

50. White NM, Chow TF, Mejia-Guerrero S, Diamandis M, Rofael Y, Faragalla H, Mankaruous M, Gabril M, Girgis A, Yousef GM. Three dysregulated miRNAs control kallikrein 10 expression and cell proliferation in ovarian cancer. Br J Cancer. 2010; 102:1244–53. https://doi.org/10.1038/sj.bjc.6605634 PMID:20354523

51. Woo HH, László CF, Greco S, Chambers SK. Regulation of colony stimulating factor-1 expression and ovarian cancer cell behavior *in vitro* by miR-128 and miR-152. Mol Cancer. 2012; 11:58. https://doi.org/10.1186/1476-4598-11-58 PMID:22909061

52. Ma H, Tian T, Liang S, Liu X, Shen H, Xia M, Liu X, Zhang W, Wang L, Chen S, Yu L. Estrogen receptor-mediated miR-486-5p regulation of OLFM4 expression in ovarian cancer. Oncotarget. 2016; 7:10594–605. https://doi.org/10.18632/oncotarget.7236 PMID:26871282

53. Wang Y, Bao W, Liu Y, Wang S, Xu S, Li X, Li Y, Wu S. miR-98-5p contributes to cisplatin resistance in epithelial ovarian cancer by suppressing miR-152 biogenesis via targeting Dicer1. Cell Death Dis. 2018; 9:447. https://doi.org/10.1038/s41419-018-0390-7 PMID:29670086

54. Youssef YM, White NM, Grigull J, Krizova A, Samy C, Mejia-Guerrero S, Evans A, Yousef GM. Accurate molecular classification of kidney cancer subtypes using microRNA signature. Eur Urol. 2011; 59:721–30. https://doi.org/10.1016/j.eururo.2011.01.004 PMID:21272993

55. Murria Estal R, Palanca Suela S, de Juan Jiménez I, Egoavil Rojas C, García-Casado Z, Juan Fita MJ, Sánchez Heras AB, Segura Huerta A, Chirivella González I, Sánchez-Izquierdo D, Llop García M, Barragán González E, Bolufer Gilabert P. MicroRNA signatures in hereditary breast cancer. Breast Cancer Res Treat. 2013; 142:19–30. https://doi.org/10.1007/s10549-013-2723-7 PMID:24129975

56. Yao Y, Suo AL, Li ZF, Liu LY, Tian T, Ni L, Zhang WG, Nan KJ, Song TS, Huang C. MicroRNA profiling of human gastric cancer. Mol Med Rep. 2009; 2:963–70. https://doi.org/10.3892/mmr_00000199 PMID:21475928

57. Ogawa T, Saiki Y, Shiga K, Chen N, Fukushige S, Sunamura M, Nagase H, Hashimoto S, Matsuura K, Saijo S, Kobayashi T, Horii A. miR-34a is downregulated in cis-diamminedichloroplatinum treated sinonasal squamous cell carcinoma patients with poor prognosis. Cancer Sci. 2012; 103:1737–43. https://doi.org/10.1111/j.1349-7006.2012.02338.x PMID:22624980

58. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, Calin GA, Ménard S, Croce CM. MicroRNA signatures in human ovarian cancer. Cancer Res. 2007; 67:8699–707.

https://doi.org/10.1158/0008-5472.CAN-07-1936 PMID:17875710

59. Prahm KP, Høgdall C, Karlsen MA, Christensen IJ, Novotny GW, Høgdall E. Identification and validation of potential prognostic and predictive miRNAs of epithelial ovarian cancer. PLoS One. 2018; 13:e0207319. https://doi.org/10.1371/journal.pone.0207319 PMID:30475821

60. Vapnik VN. An overview of statistical learning theory. IEEE Trans Neural Netw. 1999; 10:988–99. https://doi.org/10.1109/72.788640 PMID:18252602

61. Chang CC, Lin CJ. LIBSVM: A library for support vector machines. ACM Trans Intell Syst Technol. 2011; 2:1–27. https://doi.org/10.1145/1961189.1961199

62. Papadopoulos GL, Alexiou P, Maragkakis M, Reczko M, Hatzigeorgiou AG. DIANA-mirPath: Integrating human and mouse microRNAs in pathways. Bioinformatics. 2009; 25:1991–93. https://doi.org/10.1093/bioinformatics/btp299 PMID:19435746

63. An O, Pendino V, D'Antonio M, Ratti E, Gentilini M, Ciccarelli FD. NCG 4.0: the network of cancer genes in the era of massive mutational screenings of cancer genomes. Database (Oxford). 2014; 2014:bau015. https://doi.org/10.1093/database/bau015 PMID:24608173

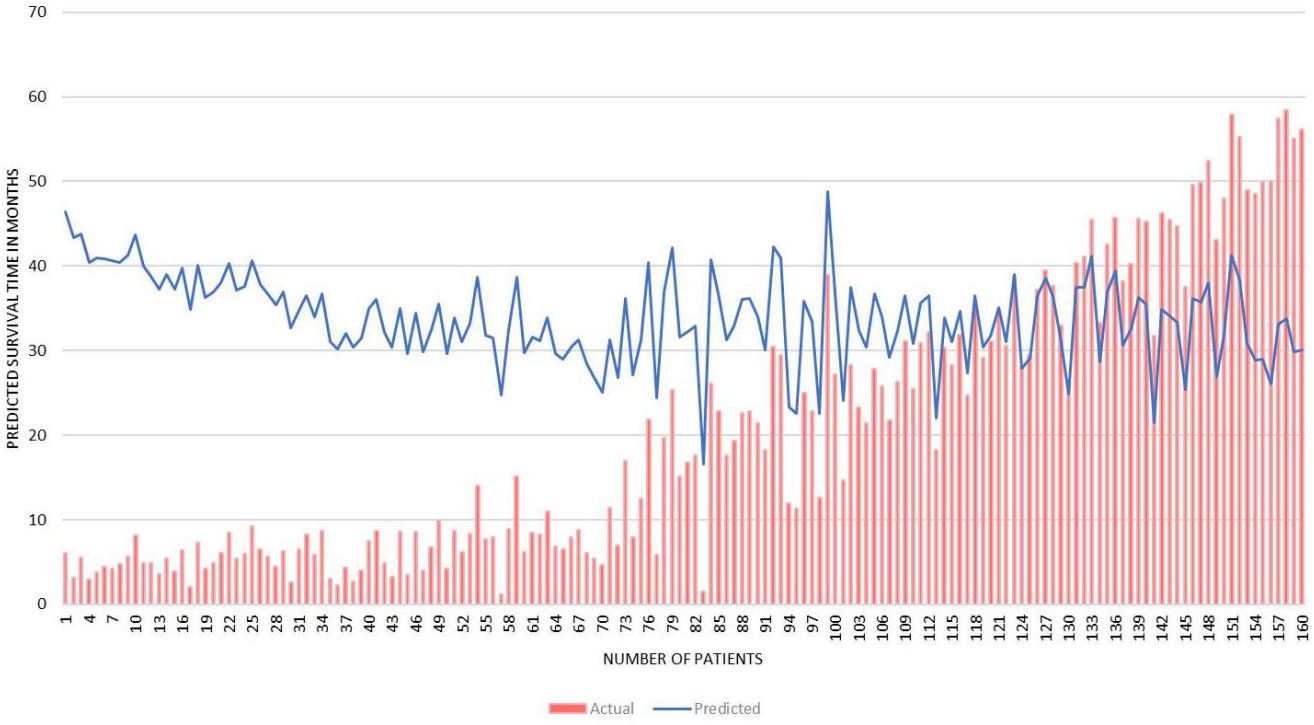
64. Hsu SD, Lin FM, Wu WY, Liang C, Huang WC, Chan WL, Tsai WT, Chen GZ, Lee CJ, Chiu CM, Chien CH, Wu MC, Huang CY, et al. miRTarBase: a database curates experimentally validated microRNA-target interactions. Nucleic Acids Res. 2011; 39:D163–69. https://doi.org/10.1093/nar/gkq1107 PMID:21071411

65. Safran M, Dalah I, Alexander J, Rosen N, Iny Stein T, Shmoish M, Nativ N, Bahir I, Doniger T, Krug H, Sirota-Madi A, Olender T, Golan Y, et al. GeneCards Version 3: the human gene integrator. Database (Oxford). 2010; 2010:baq020. https://doi.org/10.1093/database/baq020 PMID:20689021

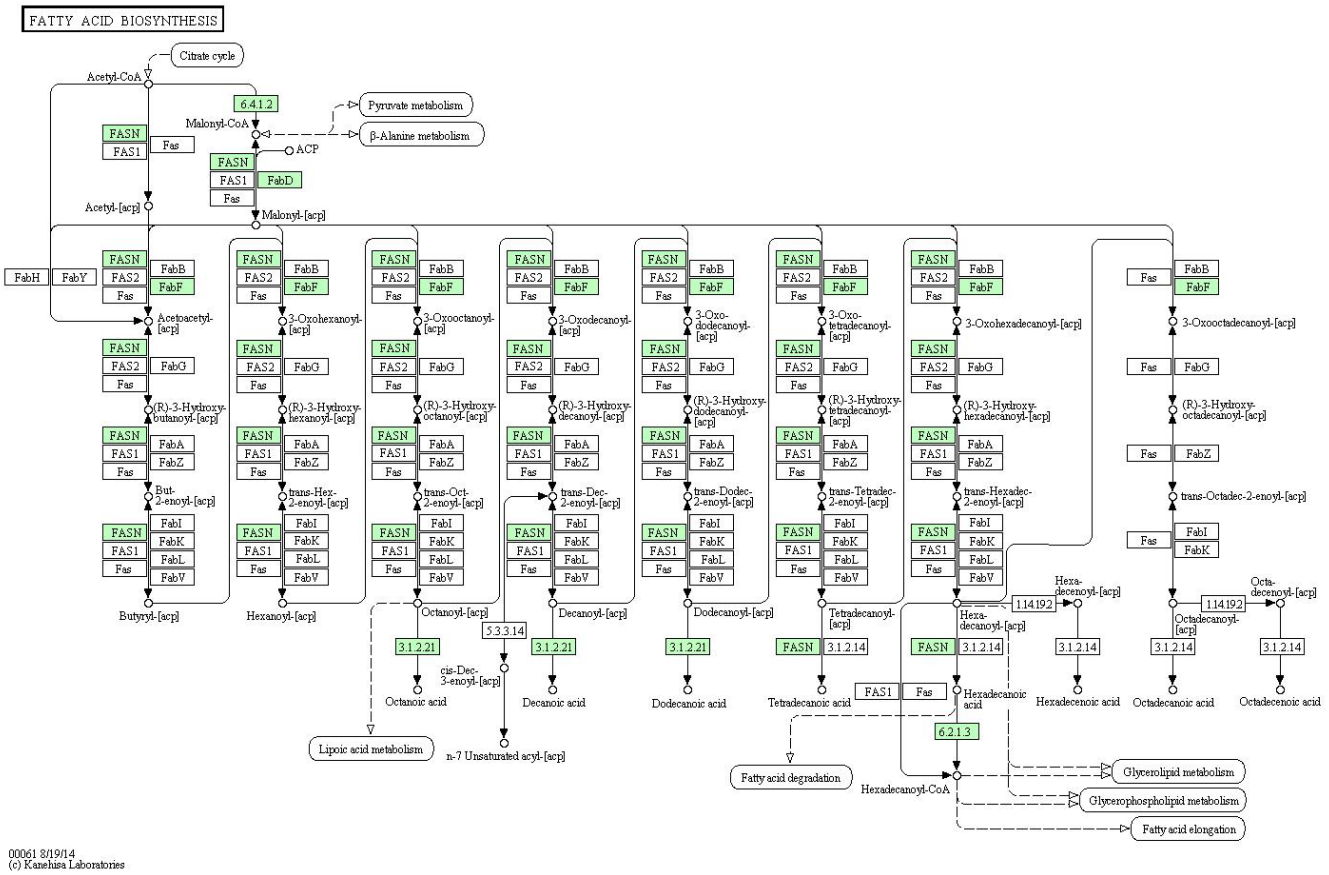
66. Wang Y, Zhao S, Zhu L, Zhang Q, Ren Y. MiR-19a negatively regulated the expression of PTEN and promoted the growth of ovarian cancer cells. Gene. 2018; 670:166–73. https://doi.org/10.1016/j.gene.2018.05.063 PMID:29783075

SUPPLEMENTARY MATERIALS

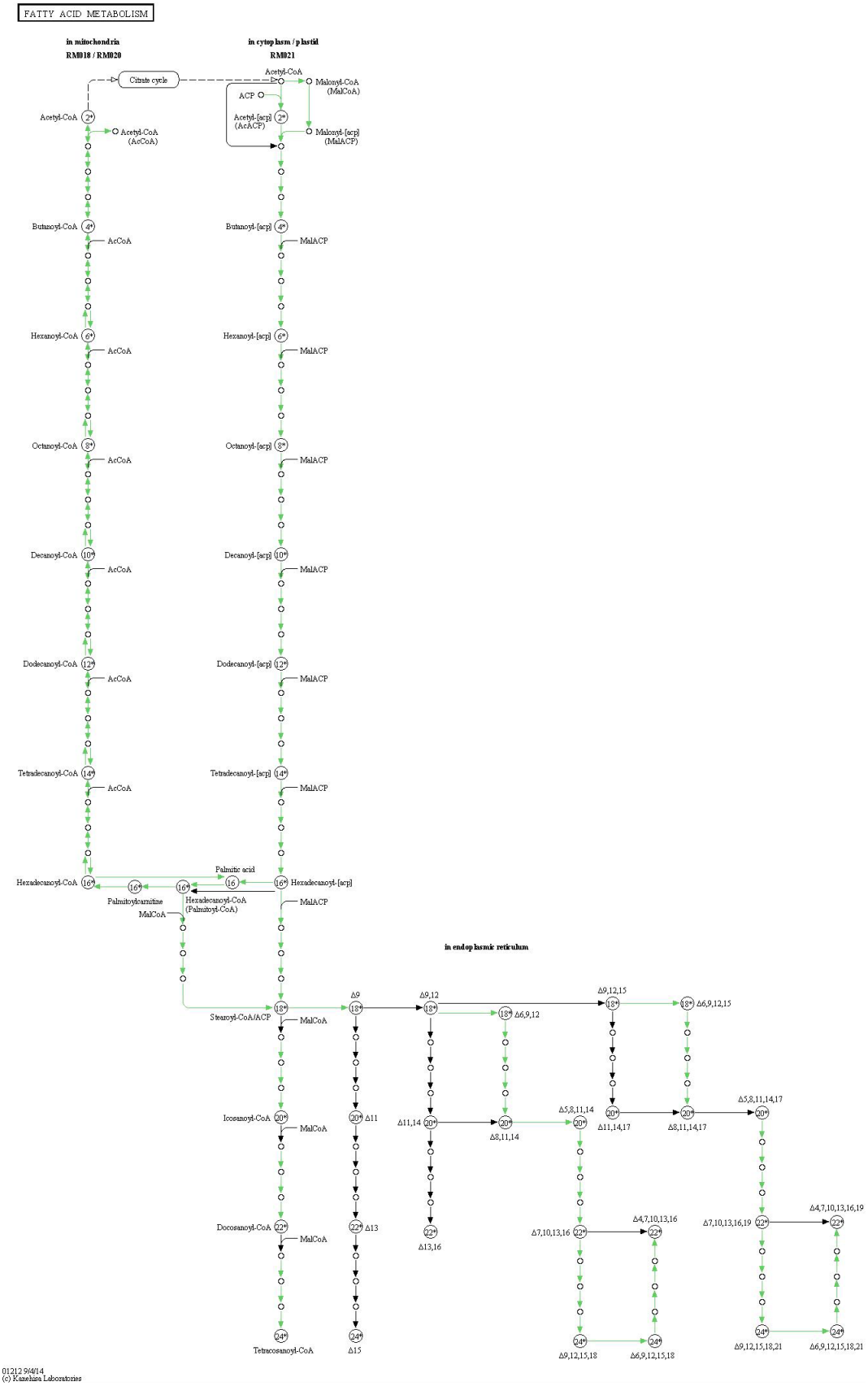
Supplementary Figures



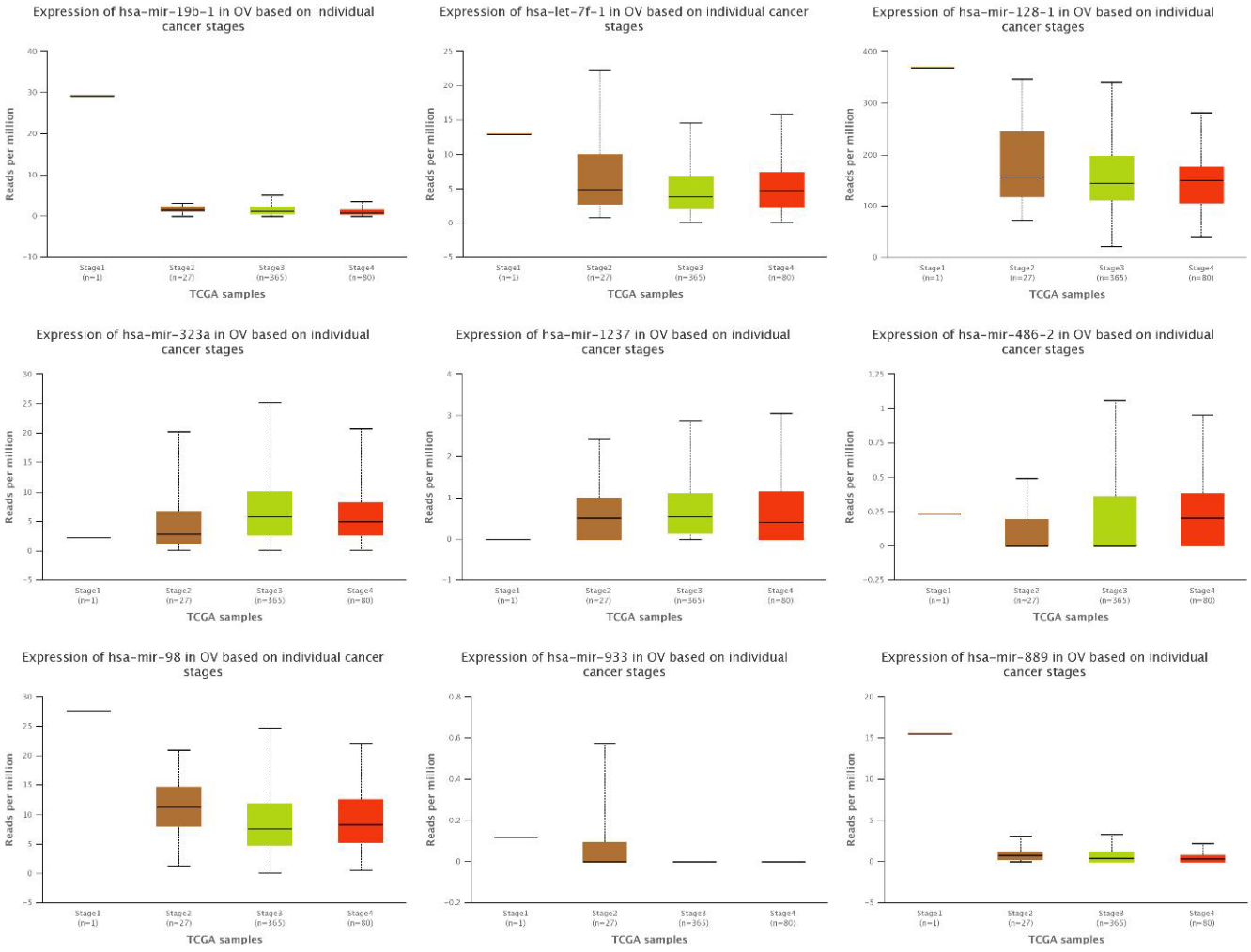
Supplementary Figure 1. The validation of OV-SURV on an independent cohort of 160 living patients with ovarian cancer. Predicted survival time is longer than the follow-up time for the first 123 patients (1–123).



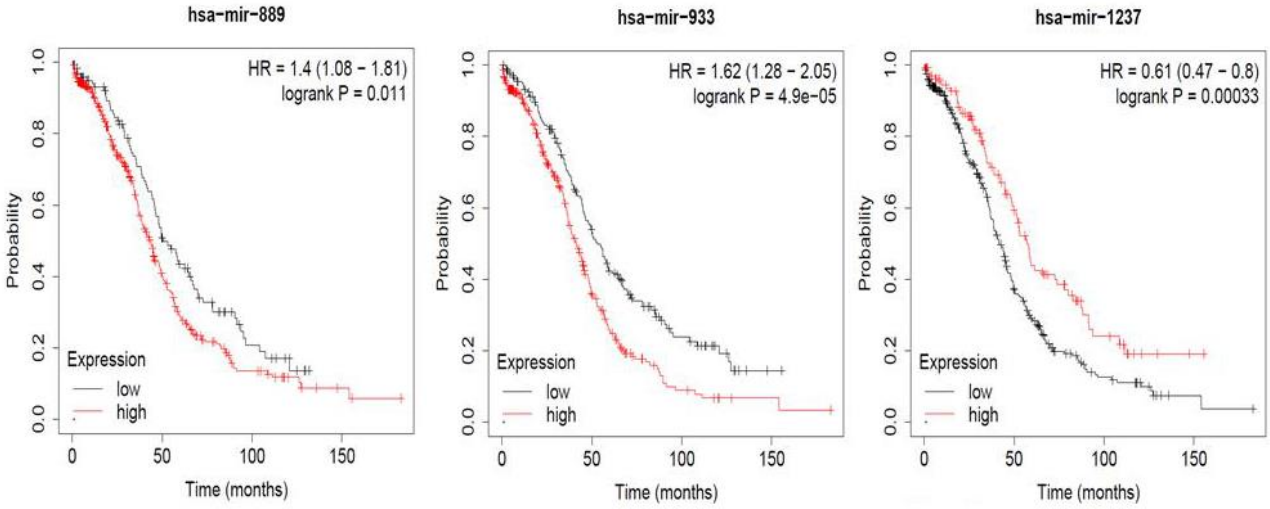
Supplementary Figure 2. Fatty acid biosynthesis flow diagram. Obtained from the KEGG pathway database.



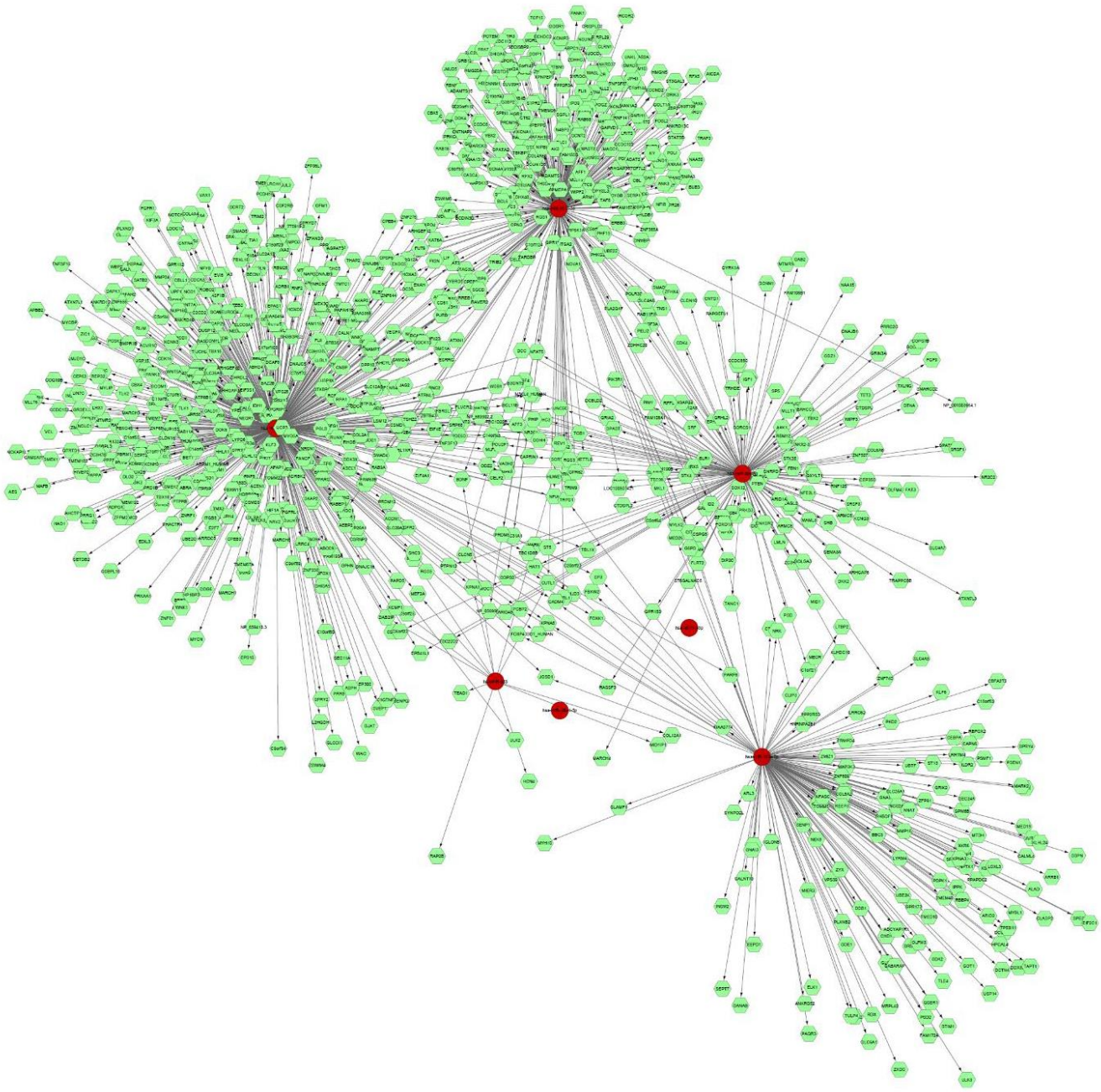
Supplementary Figure 3. Fatty acid metabolism flow diagram. Obtained from the KEGG pathway database.



Supplementary Figure 4. Box plot showing relative expression of top ranked miRNAs across stages of patients with ovarian cancer.



Supplementary Figure 5. Kaplan–Meier plots of hsa-miR-889, hsa-miR-933, and hsa-miR-1237 for the high-expression and low-expression groups of the ovarian cancer cohort.



Supplementary Figure 6. miRNA-gene target network. miRNAs are shown in red colored circles; edges represent target genes in green colored hexagons. There are 1116 predicted interactions in the network using TargetScan.

Supplementary Tables

Supplementary Table 1. Top 10 ranked miRNAs involved in KEGG pathways.

KEGG pathway	No. of genes	no. of miRNAs	p-value
TGF-beta signaling pathway	16	5	3.289E-06
Proteoglycans in cancer	26	4	7.935E-05
Sphingolipid metabolism	10	3	0.0001055
Hippo signaling pathway	24	4	0.0005239
Adherens junction	13	5	0.0094746
Signaling pathways regulating pluripotency of stem cells	21	5	0.0106464
Lysine degradation	7	3	0.0191976
Cell cycle	18	5	0.0261571
Endocytosis	24	5	0.0261571
Regulation of actin cytoskeleton	26	5	0.0261571
ECM-receptor interaction	11	5	0.0315672
Glioma	11	4	0.0419673
FoxO signaling pathway	20	4	0.0498515

Supplementary Table 2. KEGG pathway analysis of the miRNA signature.

KEGG pathway	Genes	miRNAs	p-value
TGF-beta signaling pathway	57	26	2.553E-11
Axon guidance	76	26	5.697E-07
Hippo signaling pathway	87	27	8.597E-07
Renal cell carcinoma	46	26	1.388E-06
Signaling pathways regulating pluripotency of stem cells	87	30	2.115E-06
Adherens junction	46	20	4.679E-06
Proteoglycans in cancer	108	31	5.515E-06
Fatty acid metabolism	22	18	1.541E-05
Prion diseases	14	17	2.916E-05
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	43	24	6.834E-05
Thyroid hormone signaling pathway	66	27	0.0004487
Pathways in cancer	212	35	0.0004487
Rap1 signaling pathway	113	30	0.0007618
Estrogen signaling pathway	54	26	0.0013402
Fatty acid biosynthesis	5	8	0.0017506
Lysine degradation	27	27	0.0019085
Glutamatergic synapse	62	31	0.0031419
Bacterial invasion of epithelial cells	45	28	0.0034732
FoxO signaling pathway	74	32	0.0053876
Circadian rhythm	23	27	0.0058225
Transcriptional misregulation in cancer	93	32	0.0058225
Focal adhesion	110	32	0.0073101
Dorso-ventral axis formation	20	18	0.0075923
Mucin type O-Glycan biosynthesis	16	15	0.0090565
Endocytosis	110	30	0.0093516

Oxytocin signaling pathway	83	31	0.0125495
Prostate cancer	51	31	0.0144126
Fatty acid degradation	20	15	0.0146739
Wnt signaling pathway	72	30	0.0146739
Platelet activation	71	30	0.0148846
Shigellosis	38	20	0.0162063
mRNA surveillance pathway	50	23	0.0162063
Thyroid hormone synthesis	36	26	0.0162063
Cell cycle	64	27	0.0162063
Neurotrophin signaling pathway	65	31	0.0162063
cGMP-PKG signaling pathway	86	34	0.0162063
Morphine addiction	44	26	0.0188883
Ubiquitin mediated proteolysis	70	28	0.0253401
Adrenergic signaling in cardiomyocytes	74	30	0.026999
Chronic myeloid leukemia	41	25	0.0277362
Regulation of actin cytoskeleton	108	31	0.0277362
Vasopressin-regulated water reabsorption	26	24	0.028921
T cell receptor signaling pathway	56	24	0.0352566
Glioma	35	28	0.0352566
Phosphatidylinositol signaling system	43	29	0.0352566
Protein processing in endoplasmic reticulum	83	32	0.0354841
Prolactin signaling pathway	39	24	0.0360174
GABAergic synapse	42	27	0.0360174
PI3K-Akt signaling pathway	167	35	0.0360174
RNA transport	85	31	0.0375067
Pancreatic cancer	37	27	0.041276
Melanoma	40	29	0.0430789
cAMP signaling pathway	100	34	0.0455181
Choline metabolism in cancer	55	28	0.0488212

Supplementary Table 3. GO analysis of top 10 ranked miRNAs involved in biological process, cellular components and molecular functions (p<0.05).

	GO category	miRNAs	Genes	p-value
Biological process	gene expression	3	82	1e-325
	biosynthetic process	4	360	1e-325
	cellular nitrogen compound metabolic process	4	419	1e-325
	symbiosis, encompassing mutualism through parasitism	3	68	2.787E-14
	viral process	3	62	3.15E-13
	cellular protein modification process	4	209	8.606E-13
	transcription, DNA-templated	2	170	3.92E-06
	catabolic process	2	131	1.38E-05
	response to stress	2	129	0.0002111
	nucleobase-containing compound catabolic process	2	71	0.0003728
	membrane organization	3	55	0.0005988
	small molecule metabolic process	2	138	0.0008035
	mitotic cell cycle	1	27	0.0017244
	cell death	2	62	0.0026435
	Fc-epsilon receptor signaling pathway	3	23	0.0033008
	neurotrophin TRK receptor signaling pathway	4	32	0.0034551
	mRNA metabolic process	2	23	0.0045806
	cellular component assembly	1	64	0.0061575
	DNA metabolic process	1	47	0.0103647
	macromolecular complex assembly	1	47	0.0109222
	viral life cycle	2	16	0.0129594
	RNA metabolic process	2	24	0.023756
	cellular protein metabolic process	2	31	0.0397643
Cellular components	organelle	5	834	<1e-325
	cytosol	4	248	4.462E-13
	protein complex	4	308	2.554E-11
	nucleoplasm	3	118	9.938E-11
	microtubule organizing center	1	36	0.004196
Molecular functions	ion binding	4	447	<1e-325
	RNA binding	4	192	2.22E-15
	enzyme binding	3	109	2.094E-08
	poly(A) RNA binding	2	124	1.168E-07
	protein binding transcription factor activity	2	46	4.346E-05
	nucleic acid binding transcription factor activity	2	63	0.0060971

Supplementary Table 4. GO analysis of the miRNA signature.

GO category	Genes	miRNAs	p-value
mRNA metabolic process	92	6	1.34E-19
cellular protein metabolic process	149	7	3.2E-19
nucleic acid binding transcription factor activity	264	8	3.65E-19
nucleobase-containing compound catabolic process	279	8	4.5E-19
Fc-epsilon receptor signaling pathway	69	8	7.6E-19
cell death	277	9	9.65E-19
neurotrophin TRK receptor signaling pathway	106	9	9.9E-19
membrane organization	209	9	1.201E-18
protein binding transcription factor activity	213	10	2.145E-18
response to stress	609	10	3.456E-18
macromolecular complex assembly	282	10	4.53E-18
mitotic cell cycle	188	11	5.63E-18
catabolic process	607	11	7.6E-18
cellular component assembly	399	11	9.34E-18
small molecule metabolic process	640	11	9.37E-18
enzyme binding	477	13	1.276E-17
symbiosis, encompassing mutualism through parasitism	264	13	2.1E-17
nucleoplasm	504	14	2.18E-17
viral process	234	14	4.89E-17
poly(A) RNA binding	522	14	4.89E-17
biosynthetic process	1311	16	5.437E-17
cytosol	939	17	5.647E-17
cellular protein modification process	816	17	6.54E-17
ion binding	1717	17	6.71E-17
protein complex	1226	17	8.6E-17
RNA binding	678	18	9.82E-17
gene expression	332	19	7.5E-16
cellular nitrogen compound metabolic process	1647	21	9.6E-16
organelle	3270	23	1.8E-15
RNA metabolic process	90	5	1.89E-15
transcription, DNA-templated	555	6	2.29E-14
protein complex assembly	189	4	5.08E-12
DNA metabolic process	196	5	1.25E-11
blood coagulation	118	6	1.31E-09
cytoskeletal protein binding	178	5	2.25E-09
G2/M transition of mitotic cell cycle	53	4	4.28E-08
epidermal growth factor receptor signaling pathway	55	3	6.24E-07
transcription initiation from RNA polymerase II promoter	78	5	9.1E-07
RNA splicing	87	5	1.09E-06
mRNA processing	121	4	4.43E-06
microtubule organizing center	107	3	5.08E-06
enzyme regulator activity	154	3	0.0000402
Fc-gamma receptor signaling pathway involved in phagocytosis	24	3	0.0001053

positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	17	5	0.0001221
immune system process	214	2	0.0001239
fibroblast growth factor receptor signaling pathway	46	2	0.0001266
cell cycle	163	2	0.0002052
chromatin organization	42	3	0.0017787
intrinsic apoptotic signaling pathway	28	4	0.001842
G1/S transition of mitotic cell cycle	53	3	0.002041
transcription factor binding	123	3	0.0030848
nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	25	2	0.0036671
mRNA splicing, via spliceosome	38	3	0.0059903
vesicle-mediated transport	159	2	0.0073256
innate immune response	128	4	0.0073332
viral life cycle	31	3	0.0161939
cellular lipid metabolic process	41	3	0.022398
positive regulation of transcription, DNA-templated	131	1	0.0319244
cellular component disassembly involved in execution phase of apoptosis	16	2	0.0431022

Supplementary Table 5. Expression difference of top ranked miRNAs across stages of patients with ovarian cancers.

miRNA	Comparison	p-value
hsa-miR-19b-1	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	1.635040E-01
	Stage 2 vs Stage 4	1.423760E-01
	Stage 3 vs Stage 4	6.580000E-01
hsa-let-7f-1	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	8.590100E-02
	Stage 2 vs Stage 4	2.215400E-01
	Stage 3 vs Stage 4	7.597800E-01
hsa-miR-128	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	6.593800E-01
	Stage 2 vs Stage 4	1.600640E-01
	Stage 3 vs Stage 4	9.919900E-02
hsa-miR-1978	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	N/A
	Stage 2 vs Stage 4	N/A
	Stage 3 vs Stage 4	N/A
hsa-miR-323a	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	6.543400E-01
	Stage 2 vs Stage 4	4.080600E-01
	Stage 3 vs Stage 4	1.367940E-02
hsa-miR-1237	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	4.797800E-01
	Stage 2 vs Stage 4	4.962600E-01
	Stage 3 vs Stage 4	9.581400E-01
hsa-miR-486	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	4.861000E-01

	Stage 2 vs Stage 4	2.439200E-01
	Stage 3 vs Stage 4	4.852600E-01
hsa-miR-98	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	2.358600E-01
	Stage 2 vs Stage 4	4.671800E-01
	Stage 3 vs Stage 4	3.117600E-01
hsa-miR-933	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	5.775000E-01
	Stage 2 vs Stage 4	7.479000E-01
	Stage 3 vs Stage 4	N/A
hsa-miR-889	Stage 1 vs Stage 2,3&4	N/A
	Stage 2 vs Stage 3	4.949600E-01
	Stage 2 vs Stage 4	2.817400E-01
	Stage 3 vs Stage 4	6.191800E-02

Supplementary Table 6. Top 10 ranked miRNAs and experimentally validated evidences across various cancer types.

Rank	miRNA	Cancer type	Source
1	hsa-miR-19b	ovarian cancer breast cancer lung cancer	[1–3]
2	hsa-let-7f	ovarian cancer glioma Breast cancer	[4–6]
3	hsa-miR-323	ovarian cancer prostate cancer glioblastoma	[7–9]
4	hsa-miR-1978	endometriosis Breast cancer	[10, 11]
5	hsa-miR-128	ovarian cancer colorectal cancer	[12–14]
6	hsa-miR-1237	Gastric cancer Gastric cancer	[15]
7	hsa-miR-486	ovarian cancer oral cancer gastric Adenocarcinoma	[16–18]
8	hsa-miR-98	ovarian cancer pancreatic ductal adenocarcinoma	[19, 20]
9	hsa-miR-933	Breast cancer	[21]
10	hsa-miR-889	cervical cancer colorectal cancer	[22, 23]

Supplementary Table 7. Prediction list of the identified miRNA signature associated with ovarian cancer based on known associations in dbDEMC 2.0 and HMDD v3.0 databases.

Rank	MiRNA	Cancer type	Expression status	Experiment ID	LogFC
1	hsa-miR-19b	ovarian cancer	up	EXP00327	0.20
2	hsa-let-7f	ovarian cancer	down	EXP00259	-0.93
3	hsa-miR-323	-	-	-	-
4	hsa-miR-1978	-	-	-	-
5	hsa-miR-128	ovarian cancer	up	EXP00260	1.44
6	hsa-miR-1237	ovarian cancer	up	EXP00327	0.48
7	hsa-miR-486	ovarian cancer	up	-	-
8	hsa-miR-98	ovarian cancer	down	EXP00259	-1.07
9	hsa-miR-933	-	-	-	-
10	hsa-miR-889	ovarian cancer	down	EXP00327	-0.41
11	hsa-miR-301b	ovarian cancer	up	EXP00301	1.98
12	hsa-miR-514	ovarian cancer	down	EXP00327	-0.25
13	hsa-miR-935	ovarian cancer	up	EXP00260	0.86
14	hsa-miR-653	ovarian cancer	down	EXP00260	-0.21
15	hsa-miR-1251	ovarian cancer	down	EXP00301	-2.78
16	hsa-miR-616	-	-	-	-
17	hsa-miR-662	ovarian cancer	up	EXP00327	0.49
18	hsa-miR-182	ovarian cancer	up	EXP00259	5.23
19	hsa-miR-1245	-	-	-	-
20	hsa-miR-200c	ovarian cancer	up	EXP00260	4.13
21	hsa-miR-34a	ovarian cancer	down	EXP00260	-0.16
22	hsa-miR-187	ovarian cancer	up	EXP00260	3.92
23	hsa-miR-190	-	-	-	-
24	hsa-miR-342	-	-	-	-
25	hsa-miR-513a	-	-	-	-
26	hsa-miR-146b	ovarian cancer	up	29518404	-
27	hsa-miR-1197	ovarian cancer	down	EXP00327	-0.24
28	hsa-miR-577	-	-	-	-
29	hsa-miR-185	ovarian cancer	down	23318422	-
30	hsa-miR-212	ovarian cancer	down	EXP00260	-1.71
31	hsa-miR-874	ovarian cancer	down	EXP00260	-2.11
32	hsa-miR-1304	ovarian cancer	up	EXP00327	0.25
33	hsa-miR-106b	ovarian cancer	up	EXP00260	1.55
34	hsa-miR-31	ovarian cancer	up	EXP00327	0.26
35	hsa-miR-320d	ovarian cancer	down	EXP00259	-0.81
36	hsa-miR-1295	-	-	-	-
37	hsa-miR-664	ovarian cancer	up	EXP00260	1.09
38	hsa-let-7b	ovarian cancer	down	EXP00327	-0.29
39	hsa-miR-24	ovarian cancer	up	EXP00260	0.55

Supplementary Table 8. Clinical information of independent test cohort.

Race	Asian	11
	black or african american	5
	NA	8
	White	136
Age in years (average)	56.76	
Cancer stage	Stage I	1
	Stage II	10
	Stage III	127
	Stage IV	22
Ethnicity	hispanic or latino	3
	Not hispanic or latino	101
	NA	56
Follow-up time in days (average)	454.27	

Supplementary References

1. Liu M, Yang R, Urrehman U, Ye C, Yan X, Cui S, Hong Y, Gu Y, Liu Y, Zhao C, Yan L, Zhang CY, Liang H, Chen X. MiR-19b suppresses PTPRG to promote breast tumorigenesis. Oncotarget. 2016; 7:64100–08. https://doi.org/10.18632/oncotarget.11799 PMID:27602768

2. Penyige A, Márton É, Soltész B, Szilágyi-Bónizs M, Póka R, Lukács J, Széles L, Nagy B. Circulating miRNA Profiling in Plasma Samples of Ovarian Cancer Patients. Int J Mol Sci. 2019; 20:4533. https://doi.org/10.3390/ijms20184533 PMID:31540229

3. Bulgakova O, Zhabayeva D, Kussainova A, Pulliero A, Izzotti A, Bersimbaev R. miR-19 in blood plasma reflects lung cancer occurrence but is not specifically associated with radon exposure. Oncol Lett. 2018; 15:8816–24. https://doi.org/10.3892/ol.2018.8392 PMID:29805621

4. White NM, Chow TF, Mejia-Guerrero S, Diamandis M, Rofael Y, Faragalla H, Mankaruous M, Gabril M, Girgis A, Yousef GM. Three dysregulated miRNAs control kallikrein 10 expression and cell proliferation in ovarian cancer. Br J Cancer. 2010; 102:1244–53. https://doi.org/10.1038/sj.bjc.6605634 PMID:20354523

5. Yan S, Han X, Xue H, Zhang P, Guo X, Li T, Guo X, Yuan G, Deng L, Li G. Let-7f Inhibits Glioma Cell Proliferation, Migration, and Invasion by Targeting Periostin. J Cell Biochem. 2015; 116:1680–92. https://doi.org/10.1002/jcb.25128 PMID:25735962

6. Elghoroury EA, ElDine HG, Kamel SA, Abdelrahman AH, Mohammed A, Kamel MM, Ibrahim MH. Evaluation of miRNA-21 and miRNA Let-7 as Prognostic Markers in Patients With Breast Cancer. Clin Breast Cancer. 2018; 18:e721–26. https://doi.org/10.1016/j.clbc.2017.11.022 PMID:29292183

7. Vaksman O, Tropé C, Davidson B, Reich R. Exosome-derived miRNAs and ovarian carcinoma progression. Carcinogenesis. 2014; 35:2113–20. https://doi.org/10.1093/carcin/bgu130 PMID:24925027

8. Gao Q, Yao X, Zheng J. MiR-323 Inhibits Prostate Cancer Vascularization Through Adiponectin Receptor. Cell Physiol Biochem. 2015; 36:1491–98. https://doi.org/10.1159/000430313 PMID:26160610

9. Shahar T, Granit A, Zrihan D, Canello T, Charbit H, Einstein O, Rozovski U, Elgavish S, Ram Z, Siegal T, Lavon I. Expression level of miRNAs on chromosome 14q32.31 region correlates with tumor aggressiveness and survival of glioblastoma patients. J Neurooncol. 2016; 130:413–22. https://doi.org/10.1007/s11060-016-2248-0 PMID:27573219

10. Suryawanshi S, Vlad AM, Lin HM, Mantia-Smaldone G, Laskey R, Lee M, Lin Y, Donnellan N, Klein-Patel M, Lee T, Mansuria S, Elishaev E, Budiu R, et al. Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. Clin Cancer Res. 2013; 19:1213–24. https://doi.org/10.1158/1078-0432.CCR-12-2726 PMID:23362326

11. Fix LN, Shah M, Efferth T, Farwell MA, Zhang B. MicroRNA expression profile of MCF-7 human breast

cancer cells and the effect of green tea polyphenon-60. Cancer Genomics Proteomics. 2010; 7:261–77. PMID:20952761

12. Li B, Chen H, Wu N, Zhang WJ, Shang LX. Deregulation of miR-128 in ovarian cancer promotes cisplatin resistance. Int J Gynecol Cancer. 2014; 24:1381–88. https://doi.org/10.1097/IGC.0000000000000252 PMID:25248111

13. Wu L, Shi B, Huang K, Fan G. MicroRNA-128 suppresses cell growth and metastasis in colorectal carcinoma by targeting IRS1. Oncol Rep. 2015; 34:2797–805. https://doi.org/10.3892/or.2015.4251 PMID:26352220

14. Liu HT, Xing AY, Chen X, Ma RR, Wang YW, Shi DB, Zhang H, Li P, Chen HF, Li YH, Gao P. MicroRNA-27b, microRNA-101 and microRNA-128 inhibit angiogenesis by down-regulating vascular endothelial growth factor C expression in gastric cancers. Oncotarget. 2015; 6:37458–70. https://doi.org/10.18632/oncotarget.6059 PMID:26460960

15. Liu J, Ma L, Wang Z, Wang L, Liu C, Chen R, Zhang J. MicroRNA expression profile of gastric cancer stem cells in the MKN-45 cancer cell line. Acta Biochim Biophys Sin (Shanghai). 2014; 46:92–99. https://doi.org/10.1093/abbs/gmt135 PMID:24384510

16. Nakamura N, Terai Y, Nunode M, Kokunai K, Konishi H, Taga S, Nakamura M, Yoo M, Hayashi M, Yamashita Y, Ohmichi M. The differential expression of miRNAs between ovarian endometrioma and endometriosis-associated ovarian cancer. J Ovarian Res. 2020; 13:51. https://doi.org/10.1186/s13048-020-00652-5 PMID:32359364

17. Chou ST, Peng HY, Mo KC, Hsu YM, Wu GH, Hsiao JR, Lin SF, Wang HD, Shiah SG. MicroRNA-486-3p functions as a tumor suppressor in oral cancer by targeting DDR1. J Exp Clin Cancer Res. 2019; 38:281. https://doi.org/10.1186/s13046-019-1283-z PMID:31253192

18. Chen H, Ren C, Han C, Wang D, Chen Y, Fu D. Expression and prognostic value of miR-486-5p in patients with gastric adenocarcinoma. PLoS One. 2015; 10:e0119384. https://doi.org/10.1371/journal.pone.0119384 PMID:25793394

19. Wang Y, Bao W, Liu Y, Wang S, Xu S, Li X, Li Y, Wu S. miR-98-5p contributes to cisplatin resistance in epithelial ovarian cancer by suppressing miR-152 biogenesis via targeting Dicer1. Cell Death Dis. 2018; 9:447. https://doi.org/10.1038/s41419-018-0390-7 PMID:29670086

20. Fu Y, Liu X, Chen Q, Liu T, Lu C, Yu J, Miao Y, Wei J. Downregulated miR-98-5p promotes PDAC proliferation and metastasis by reversely regulating MAP4K4. J Exp Clin Cancer Res. 2018; 37:130. https://doi.org/10.1186/s13046-018-0807-2 PMID:29970191

21. Tanman Ü, Yangın S, Cansaran-Duman D. Determination of Dysregulated miRNA Expression Levels by qRT-PCR after the Application of Usnic Acid to Breast Cancer. Anticancer Agents Med Chem. 2020; 20:548–58. https://doi.org/10.2174/1871520619666190923163552 PMID:31549595

22. Sun Y, Cheng Y, Zhang Y, Han K. MicroRNA-889-3p targets FGFR2 to inhibit cervical cancer cell viability and invasion. Exp Ther Med. 2019; 18:1440–48. https://doi.org/10.3892/etm.2019.7675 PMID:31316631

23. Xiao Y, Li ZH, Bi YH. MicroRNA-889 promotes cell proliferation in colorectal cancer by targeting DAB2IP. Eur Rev Med Pharmacol Sci. 2019; 23:3326–34. https://doi.org/10.26355/eurrev_201904_17695 PMID:31081086