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Original Article

Evaluation of miRNA-derived Extracellular Vesicles as Biomarkers in Ovarian Cancer Stem Cells

Minoo Roostaie[1], Ali Esmaily[1], Hamid Taghvaei Javanshir[2,3], Maryam Arabi[1], Ali Tafti[4], Ahmad Bereimipour[5*], Habibollah Mahmoodzadeh[2*], Farimah Hadjilooei[2] and Melikasadat Hosseininejad[1]

[1]School of Medicine, Islamic Azad University Tehran Medical Branch, Tehran, Iran; [2]Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran; [3]Department of Pharmacology and Physiology, University of Rochester, Rochester, NY, USA; [4]Department of Biotechnology and Molecular Medicine, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran; [5]Department of Biological Sciences and BioDiscovery Institute, University of North Texas, Denton, TX, USA

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Abstract

Background and objectives: Ovarian cancer (OvCa) is the most deadly gynecological cancer. This study aimed to determine which genes and miRNAs play an important role in OvCa.

Methods: We accessed the Gene Expression Omnibus database and downloaded the mRNA microarray dataset. Through the use of GEO2R, we were able to collect data on differentially expressed genes (DEGs) and microRNAs (DEMs). By querying the Enrichr database, we were able to conduct functional and pathway enrichment analysis on DEGs. STRING was used to create a network of protein–protein interactions, and Cytoscape was used to display the networks. Gene Expression Profiling Interactive Analysis and The Cancer Genome Atlas were then used to conduct overall survival and clinical data analyses of hub genes. DEM target predictions were also made using miRnet. For extracellular vesicles confirmation, Exocarta and Vesiclepedia were used.

Results: There were a total of 1,778 DEGs found, and most of them were enriched for terms associated with the cell cycle, mitosis, and the ovulation cycle. There were 141 nodes used in the creation of the protein–protein interaction network. There were 10 genes with a lot of connections between them. Patients with OvCa had a shorter overall survival if they had high expression of four of the 10 genes tested: *ATF3*, *ZEB1*, *CSF1R*, and *HSPA8*. We found out that the protein–protein interaction network has a significant module. The cell cycle, extracellular matrix receptor, and cell invasion were among the enriched functions and pathways. In addition, we found a total of 20 DEMs. The hsa-let-7 family (hsa-let-15a-3p, hsa-let-18a-5p, and hsa-let-615-5p) may target ZEB1 because its expression is inversely correlated with that of *ZEB1*.

Conclusions: This study uncovered critical genes that represent promising therapeutic targets in the fight against OvCa.

Keywords: Extracellular vesicles; miRNAs; Ovarian cancer stem cells; Bioinformatics analysis.

Abbreviations: AF-EVs, ascitic fluid-derived extracellular vesicles; circRNAs, circular RNAs; cRNAs, coding RNAs; CCs, cancer cells; CD8, cluster of differentiation 8; CRC, colorectal cancer; CSCs, cancer stem cells; DEGs, differentially expressed genes; DEMs, differentially expressed miRNAs; EMT, epithelial–mesenchymal transition; EV, extracellular vesicle; *FN1*, fibronectin 1; GCSCs, gastric cancer stem cells; HNSCC, head and neck squamous cell carcinoma; KCNQ1OT1, KCNQ1 opposite strand/antisense transcript 1; miRNAs, micro RNAs; MMP, matrix metalloproteinase; OCAMs, ovarian cancer-associated mesothelial cells; OC-EVs, ovarian cancer extracellular vesicles; OMM, oligonucleotide miRNA mimic; OvCa, ovarian cancer; PC, prostate cancer; PD-L1, programmed death-ligand 1; *PTAL*, promoting transition-associated long noncoding RNA; *SORBS2*, sorbin and SH3 domain containing 2.

[*]**Correspondence to:** Habibollah Mahmoodzadeh, Cancer Research Center, Tehran University of Medical Sciences, Tehran 2706856, Iran. ORCID: https://orcid.org/0000-0001-6840-2645. Tel: +98-21–61192541, Fax: +98-21–88485429, E-mail: hmahmoodzadeh@tums.ac.ir; Ahmad Bereimipour, Department of Biological Sciences and BioDiscovery Institute, University of North Texas, Denton 76203, TX, USA. ORCID: https://orcid.org/0000-0002-6758-9599. Tel: +19-40-6292360, E-mail: ahmadlta@yahoo.com

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Introduction

The mortality rate associated with ovarian cancer (OvCa) is the greatest of all gynecological cancers. In 2019, there were 22,530 newly discovered cases of this disease in the United States. More than 70% of patients are diagnosed at an advanced stage (International Federation of Gynecology and Obstetrics stage III or IV), and this is largely attributable to the absence of typical symptoms as well as sensitive diagnostic methods.[1] Systemic chemotherapy

with platinum as the active ingredient is the next best treatment choice following debulking surgery and precise surgical staging. Despite recent advancements in the treatment and availability of cutting-edge medications (such as anti-angiogenesis therapies and poly(ADP-ribose) polymerase inhibitors), the 5-year survival rate is still a dismal 47%.[2] For this reason, it is of utmost relevance and urgency to better understand the process behind carcinogenesis in OvCa as well as to create innovative tools for early identification, disease monitoring, and prognostic evaluation.[3]

OvCa, like other types of carcinogenesis, is produced by mutations in a variety of genes throughout the body. Unfortunately, the mechanisms that, in the end, lead to the creation of OvCa are still not well understood.[4] Quantification of epigenetic markers such as DNA methylation and histone modifications, as well as RNA intermediates, has been the focus of a great deal of research and development over the course of the last several decades, leading to the development of a large number of high-throughput approaches. Rapid advancements in microarray technology and bioinformatics analysis have led to a better understanding of the genomic abnormalities underpinning carcinogenesis and prognosis as well as the identification of differentially expressed genes (DEGs) and functional pathways implicated in both.[3,5] This has resulted in a more accurate prediction of the likelihood of survival for patients with cancer.

A number of human solid cancers, including OvCa,[6] have been found to include cancer stem cells (CSCs) that have the potential to become tumorigenic. CSCs are thought to emerge from the cellular reprogramming that takes place throughout the process of neoplastic transformation,[7] which is a theory that is widely recognized. Cellular signaling mechanisms in the tumor microenvironment are responsible for keeping the dynamic balance between cancerous cells (CCs) and CSCs.[8] The process of differentiation of CCs enables the cells to acquire characteristics similar to those of stem cells,[9] but it also disrupts the dynamic balance that exists between the CCs and their surroundings. This equilibrium is maintained by the continuous communication that takes place between CSCs and CCs that are operating in the CSC niche. Therefore, a differential gene expression study of CCs and CSCs[10] is required in order to uncover novel molecular targets that can be exploited to improve the outcomes of OvCa patients.

In recent years, there has been a remarkable advancement in the study of extracellular vesicles (EVs) and their function in cancer.[11] EVs are classified into a few different classes according to their sizes as well as the processes that are responsible for their biogenesis. The most extensively researched major groupings include apoptotic bodies (1–5 μm), big oncosomes (1–10 μm), and various miscellaneous EV subsets.[12] The most extensively researched EVs are exosomes (40–160 nm in diameter, endosomal origin) and ectosomes (100–1,000 nm in diameter, direct budding of the plasma membrane). Because of their unique biogenesis and activities, exosomes have been the subject of the majority of scientific investigations. The coding RNAs (cRNAs) include microRNAs (miRNAs), long noncoding RNAs, and circular RNAs (circRNAs), whereas the noncoding RNAs consist of proteins, DNAs, and lipids. The term "extracellular vesicles" is used by the International Society of Extracellular Vesicles[13] to refer to diverse populations of vesicles that have been recovered from cell culture supernatants or physiological fluids. In the interest of brevity and clarity throughout this paper, we shall abbreviate "exosomes" to "EVs."

The role that EVs play in the evolution of OvCa, the spread of metastases, and the development of drug resistance is becoming more and more clear.[14] The primary concentration of EV research[15] has been on determining whether or not EVs have the potential to serve either as a diagnostic biomarker or as a therapeutic delivery strategy for OvCa. In addition, immunotherapy treatments that make use of EVs are a relatively new area of research.[16] In this study, we used an integrated *in-silico* analysis to search for novel miRNAs in EVs that have the potential to be utilized as biomarkers for the diagnosis and treatment of OvCa.

Material and methods

Locating the appropriate data set

For the purpose of this investigation, we accessed the Gene Expression Omnibus repository (https://www.ncbi.nlm.nih.gov/geo/) and extracted the pertinent data. The microarray dataset GSE64999 contains eight samples that have been tiered according to pathological criteria, moving from differentiated CSCs to undifferentiated CSCs. GPL17077 is the server that stores this information. Microarray model number 039381 for the SurePrint G3 Human GE v2 8x60K (Agilent part number: 039494) was used in this study. For the purpose of this investigation, OvCa patients were divided into two categories: those whose disease's stem cells had differentiated and those whose stem cells had not yet differentiated. Figure 1 presents some additional contextual information regarding this data store.

Feature analysis

We used information from the Gene Expression Omnibus database and the GEO2R program (https://www.ncbi.nlm.nih.gov/geo/geoR/) to compare the gene expression profiles of the differentiated and undifferentiated phases of OvCa. After that, we split the genes with high expression into separate Excel files from those with low expression. A value of $p < 0.05$ was considered to be the appropriate threshold of significance for this investigation.

Gene ontologies and the associated signaling pathways

At this point, high- and low-expressed genes were each uploaded to the Enrichr database (https://maayanlab.cloud/Enrichr/), which may be found at maayanlab.cloud. The Kyoto Encyclopedia of Genes and Genomes (accessible at https://www.genome.jp/kegg/) and the Reactome library (accessible at https://reactome.org/) were utilized to dissect the signaling pathways and to isolate them from the gene clusters. In order to conduct an analysis of the gene ontology, we made use of the Enrichr and PANTHER (http://www.pantherdb.org/) databases in conjunction with the Gene Ontology library (http://geneontology.org/). After that, with the assistance of the ShinyGO database (http://bioinformatics.sdstate.edu/go/), we drew the interaction network for the gene ontology.

Examining the relationships between different protein byproducts

We used the STRING database (https://string-db.org/) in order to acquire additional information regarding these proteins. After that, we investigated the node degree and betweenness features of the proteins that had the strongest ties to one another in terms of connectivity.

Validation of probable genes in humans

The Cancer Genome Atlas (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcg) and The Genotype-Tissue Expression (https://gtexportal.org/home/) databases were used to confirm the selected genes. The final genes

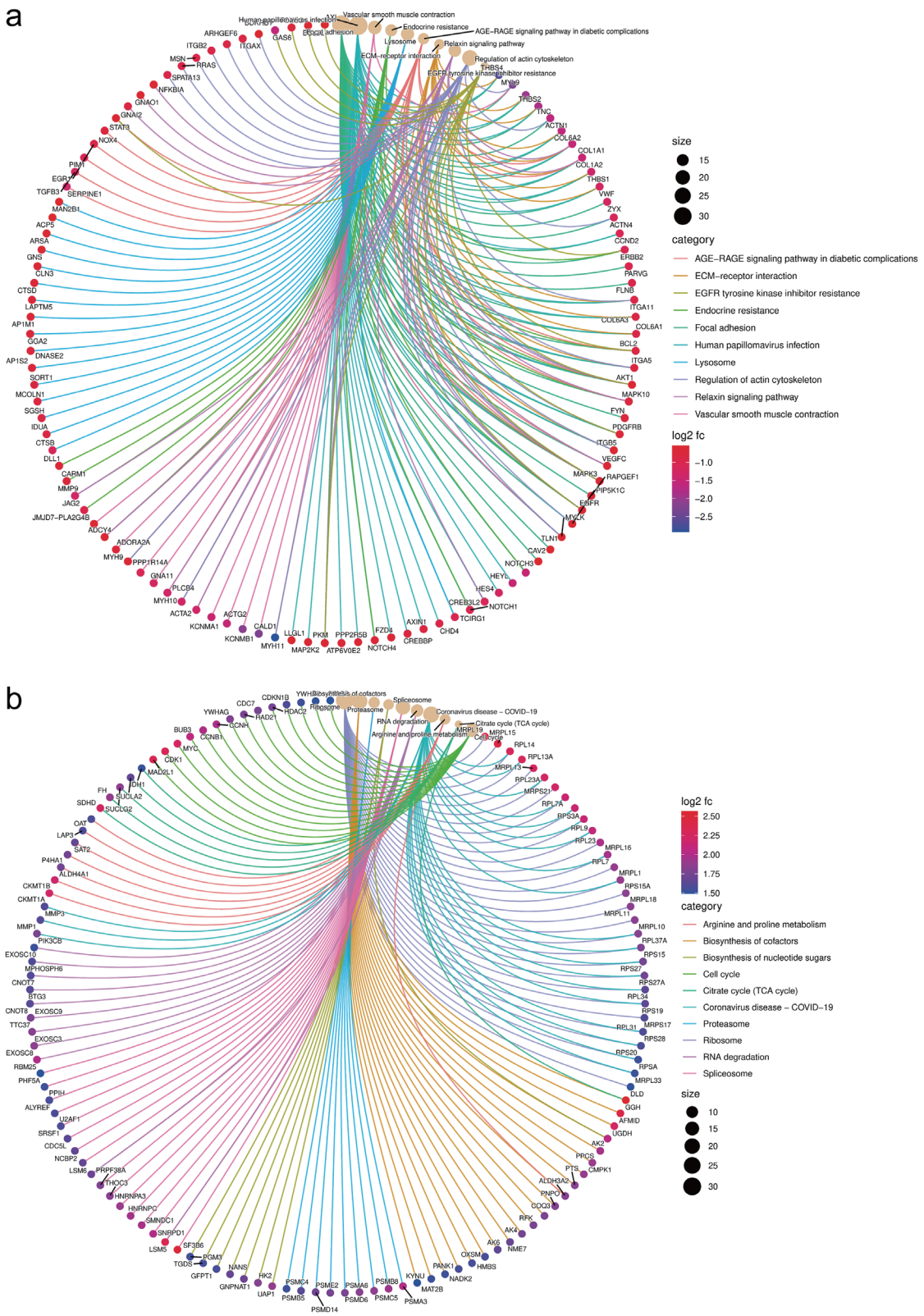

Fig. 1. Signaling pathways involved in ovarian cancer stem cells. In this figure, which is drawn as a chord chart, the signaling pathways related to the expression profile of genes with high and low expression can be seen. The color spectrum indicates the Log (fold change), and the size of the circles indicates the number of genes involved in each signaling pathway. (a) Upregulated pathways; (b) downregulated pathways.

were evaluated by Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn/). Box plots were made to display the gene expression and survival data, and the Gene Expression Profiling Interactive Analysis database was used as the foundation for these plots.

Localization of genes and their corresponding mRNAs

Initially, a miRNA-target gene network was constructed with the use of the online miRNet program (http://www.mirnet.ca). Scores based on both the degree and betweenness centrality were utilized in order to determine which miRNAs were the most important in the network. We were able to accurately predict the experimentally validated target genes of miRNAs by making use of various online tools such as miRNet, miRTarBase, and TargetScan (http://miRTarBase.mbc.nctu.edu; www.targetscan.org).

EV miRNA candidates

We conducted research using the miRNet database to ascertain whether the EVs were significant.

Results

The activation of certain pathways within OvCa stem cells by a signaling substance

The use of the Venn diagram revealed that there were 911 upregulated genes and 829 genes with a low expression. The data (Fig. 1) illustrate the pathways that are present in downregulated genes and upregulated genes. These pathways include protein targeting to the endoplasmic reticulum (30 genes), (cytosolic) ribosome (29 genes), ribosome (32 genes), and FOXP2 OE SHSY5Y HUMAN GSE100291 RNASEQ DOWN (40 genes).

Evaluation of gene ontology between OvCa and stem cells

Next, the common genes from the previous step were determined to examine their molecular functions and biological processes. Accordingly, RPS20, HEL114, LIMA1, VMP1, and RPL3 involved in FOXP2 OE, SHSY5Y HUMAN GSE100291, RNASEQ DOWN, RPS20, RPL3, RPL34, RPS15, RPL22, and RPL21 in ribosomes; and RPS20, RPL3, RPL34, RWDD1, and CPEB4 in cytoplasmic translation were observed in genes with abnormal expression. RPL5, RPL30, RPL3, RPL32, and RPL34 were involved in cytoplasmic ribosomal proteins; RPL5, RPL30, RPL3, RPL32, and RPL34 were involved in translation; RPL5, RPL30, RPL3, RPL32, and RPL21 were involved in Cap-dependent translation initiation, and RPL5, RPL30, RPL3, RPL32, and RPL34 were involved in protein metabolism for downregulated genes. In addition, CSF1R, PXN, and KDR were involved in integrins in angiogenesis; HSPA8, GADD45A, DUSP1, and ITGA2 were involved in the BDNF signaling pathway; ZEB1, ANKRD1, CYR61, and ATF3 were involved in the hypertrophy pathway; and PXN, BCAR1, and VCL were involved in the cell-to-cell adhesion signaling for upregulated genes (Fig. 2).

Protein–protein interactions in OvCa stem cells

The upregulated protein network consists of 58 nodes and 185 edges, while the downregulated protein network consists of 32 nodes and 46 edges; the proteins that interact better with other proteins are located at the center of the network. Their communication network also was mapped for low-expression genes, and their upstream and downstream elements were identified. High-expression proteins (FN1, ITGAV, ITGA5, KDR, and VCL) have 13–19 nodes, and low-expression proteins (RPL35, RPS3, RPL22, RPL39, and RPS14) have 33–34 nodes (Fig. 3).

Evaluation of over-expressed proteins in human OvCa data

After selecting the genes and proteins with a significantly abnormal performance compared to the other proteins, we decided to evaluate the protein code genes in the Gene Expression Profiling Interactive Analysis database. In general, there were different protein expression levels in OvCa patients than in healthy individuals. The stage plot also indicates that most patients have an increased expression of candidate genes. The survival rate of individuals over time is about 79% to 17%, depending on the tumor stage, which indicates the significant role of the candidate genes in this study (Fig. 4).

Nomination of suitable miRNAs related to the OvCa stem cells

Furthermore, we evaluated the candidate genes and proteins in terms of the upstream regulatory elements. A communication network between miRNAs was also drawn. hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7e-5p, hsa-mir-15a-5p, and hsa-mir-26b-5p regulated the high-expression genes, and hsa-let-7a-5p, hsa-mir-16-5p, hsa-mir-17-5p, and hsa-mir-148a-3p regulated the low-expression genes (Fig. 5).

EV miRNAs in OvCa stem cells

To find better and suitable miRNAs in blood samples, we investigated the miRNAs of EVs. hsa-let-7f-5p, hsa-let-7c-5p, hsa-let-7a-5p, hsa-mir-15a-5p, and hsa-mir-29b-3p regulated the high-expression genes, and hsa-mir-615-3p, hsa-mir-18a-5p, and hsa-mir-16-5p regulated the low-expression genes (Fig. 6).

Discussion

OvCa is a leading killer of women worldwide. When a patient's cancer returns, she may no longer respond to treatments. This is bad news because it implies that medical options are restricted. Accumulating evidence indicates that CSCs in close proximity to CCs in the bulk of the tumor are to blame. Characterizing CSCs based on their molecular fingerprints may help researchers find new therapeutic targets for the treatment of OvCa.[17,18] Differential gene expression analysis is often performed to discover genes that demonstrate abnormal expression in response to either of two sets of circumstances. This research used microarray datasets and bioinformatics methods to identify DEGs that distinguish CSCs from miRNA biomarkers. Out of 1,778 DEGs, 911 were found to be upregulated and 867 were found to be downregulated after analysis. Then, we looked into the enriched pathways in the Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and Reactome databases to check if these genes were involved in these processes. Analysis of biological processes, including cell proliferation, tissue development, and reactivity to lipids, chemicals, and organic substances, revealed that the majority of the upregulated DEGs were abundant in the cellular extracellular matrix. Meanwhile, a disproportionate number of downregulated DEGs was also implicated in biological processes that govern molecular activity. Numerous cell signaling pathways rely on downregulated DEGs, many of which are upregulated in CSCs. DEGs that are normally downregulated are frequently upregulated in CSCs. Interferon-alpha/beta signaling (*IFIT3*, *IFIT2*, *IFIT1*, *ISG15*, *IFITM1*, and *IFNAR1*), fiber elasticity (*BMP4*, *FBLN1*, *LTBP2*, and *TGF1*), and bile acid and bile salt production (*AKR1C3*, *AKR1C2*, and *AKR1C1*) were overrepresented among the upregulated DEGs. There was no significant

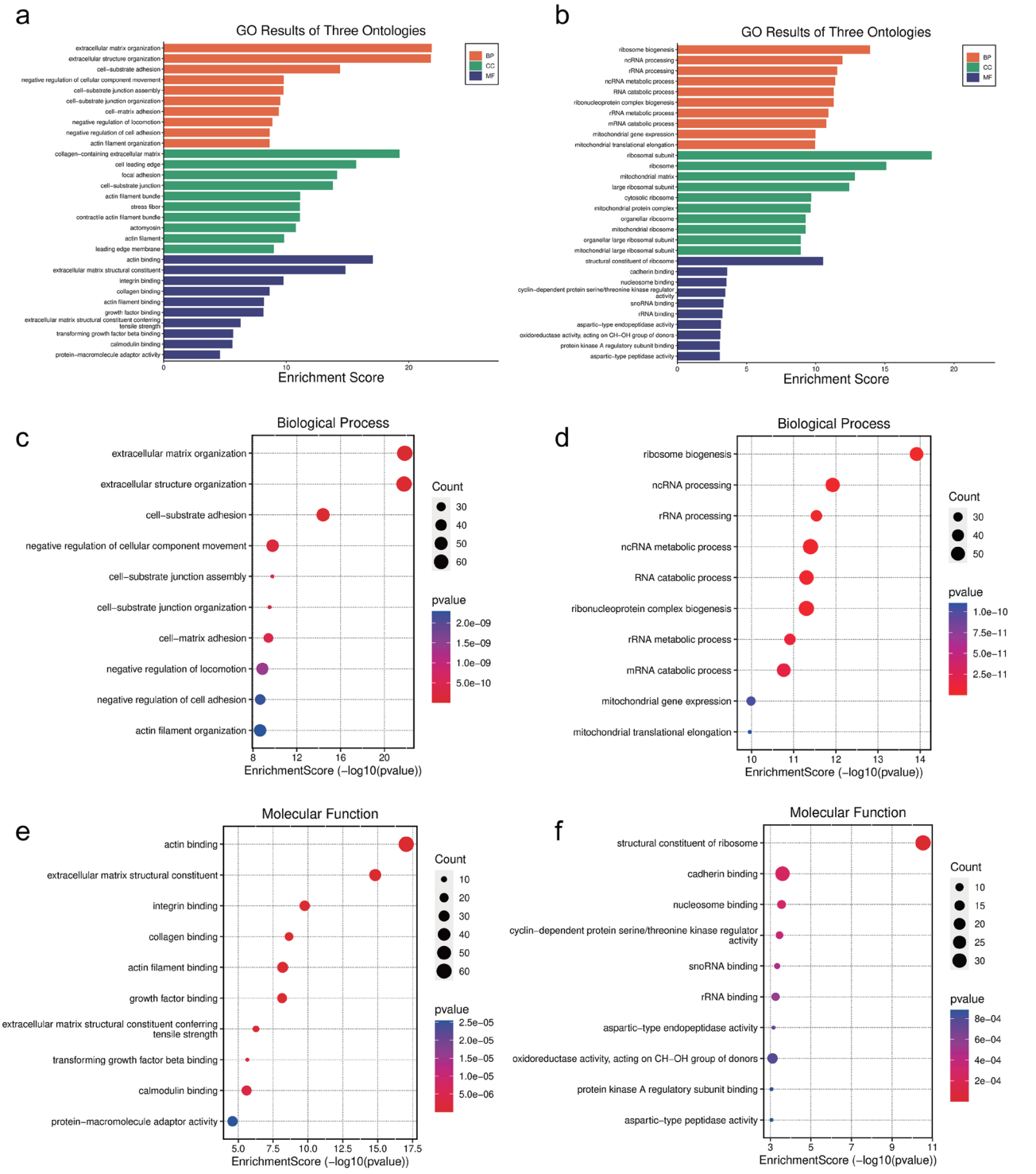

Fig. 2. Gene ontology analysis in ovarian cancer stem cells. Ontologies with high and low expression were evaluated as approach, molecular functions, or biological processes. (a–b) In the bar plots, the most important paths are mentioned in general. (c and e) In the loli plots, we pointed out more precisely the difference in the expression of genes and the number of involved genes in the molecular functions. (d and f) We investigated the genes in the biological processes.

enrichment of genes involved in the EGFR transactivation by the gastrin pathway (*PRKCA* and *EGFR*) among the genes that were repressed in CSCs. The development of individualized treatments requires an understanding of the molecular markers and signaling mechanisms that distinguish CSCs. Furthermore, reduced expression of RPL35, RPS3, RPL22, RPL39, and RPS14 proteins,

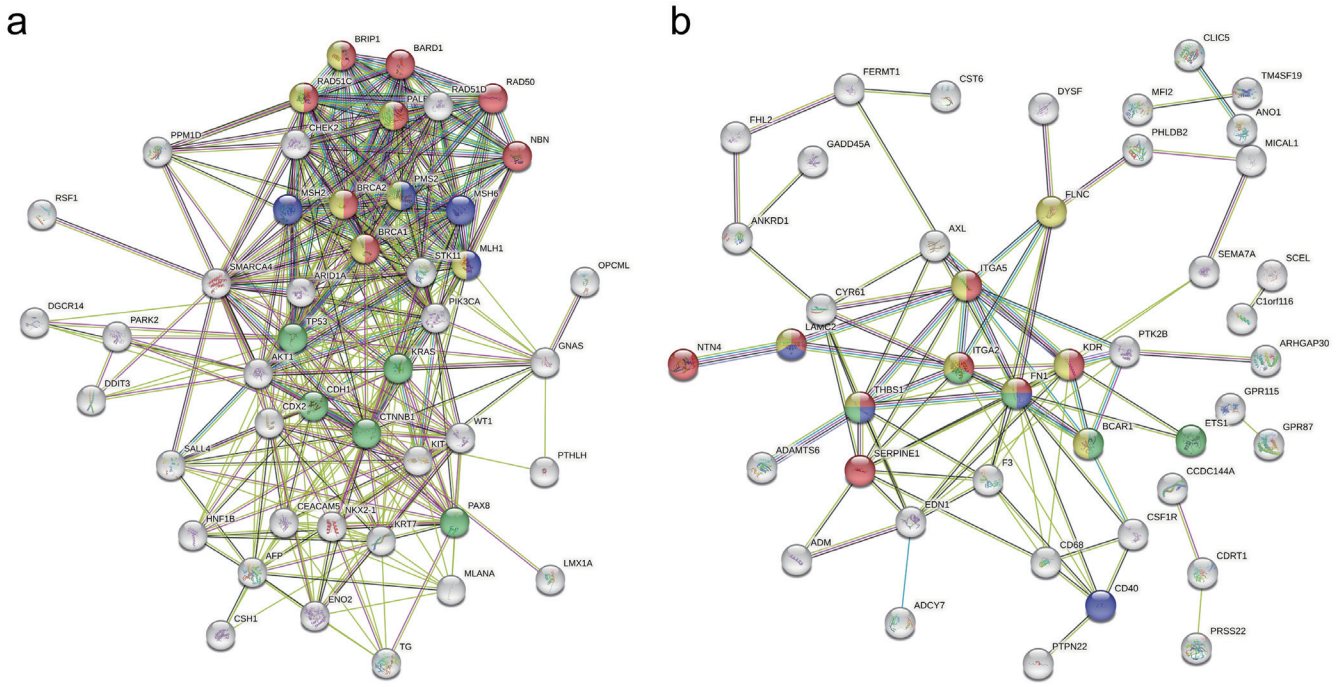

Fig. 3. Protein–protein interactions in ovarian cancer stem cells and the extracellular matrix. The proteins that were observed in the important signaling pathways of ovarian cancer stem cells were isolated and investigated in terms of the relationship between the protein products and the type of function and their interactions together. (a) Upregulated proteins; (b) downregulated proteins.

and high expression of FN1, ITGA5, KDR, and VCL proteins all played critical roles in the development and spread of OvCa. We also postulated that EVs included miRNAs from the hsa-let-7 family, including hsa-mir-15a-5p, hsa-mir-29b-3p, hsa-mir-615-3p, hsa-mir-18a-5p, and hsa-mir-16-5p.

As mediators of intercellular communication and transporters of bioactive chemicals that can alter the phenotype of target cells,[19] ovarian cancer extracellular vesicles (OC-EVs) play a pivotal role in the development of cancer. OC-EVs have been demonstrated to have unique protein signatures, in addition to the tetraspanins, heat shock, membrane proteins, antigens, and enzymes commonly found in tumor-derived EVs. Nanog, a transcription regulator involved in both the proliferation and the self-renewal of CCs and CSCs,[20] is transported by OC-EVs. Mesenchymal cells are prompted to undergo epithelial–mesenchymal transition (EMT) after receiving CD44 from OC-EVs. The E-cadherin downregulation and matrix metalloproteinase (MMP) expression stimulation that results[21] are critical for facilitating OvCa invasion and metastasis via extracellular matrix breakdown. OvCa patients have been reported to have CD24 and EpCAM on their ascitic fluid-derived EVs (AF-EVs).[22] Both of these substances aid in tumor invasion by increasing cell movement. In addition, proteins involved in drug transport or cell cycle control, such as ZBED2 and ZBTB20, were more concentrated in AF-EVs than in benign EVs, but transgelin and MARCKS were less concentrated. This was because fewer transgelin and MARCKS proteins were present in AF-EVs. Soluble L1, an adhesion molecule, is present in AF-EVs,[23] along with MT1-MMP and MMP2, both of which are membrane-type MMPs. MMP2 and urokinase-type plasminogen activator are two additional related proteins.

The *FN1* gene encodes fibronectin, a glycoprotein that exists as a dimeric form in the plasma and as a dimeric or multimeric form on the cell surface and in the extracellular matrix. Proteolytic processing is required to convert the encoded preproprotein into the functional protein. Fibronectin is involved in the adhesion and migration of cells at every stage of development, from embryogenesis to wound healing and blood clotting to host defense and metastasis. The gene contains three alternative splice sites; therefore, there is at least one transcript variant that encodes a protease-sensitive isoform. The lengths of certain variations remain unknown.[24] OvCa growth may be aided by aberrant expression of the long noncoding RNA AC004988.1, also called promoting transition-associated long noncoding RNA (*PTAL*). Although *PTAL* has been observed in OvCa, the fundamental mechanism that produces it remains unclear. In The Cancer Genome Atlas serous OvCa datasets, *PTAL* expression was found to be significantly higher in mesenchymal subtype samples compared to epithelial subtype samples. Liang and colleagues came at this conclusion. In the mesenchymal OvCa samples, *PTAL* expression was shown to positively correlate with *FN1* expression; however, both *PTAL* and *FN1* expression were found to negatively correlate with the expression of miR-101. Reducing PTAL levels also inhibited cell migration, invasion, and metastasis formation *in vitro*. Similarly, downregulation of PTAL resulted in elevated miR-101 levels, which repressed *FN1* gene expression. Specifically, PTAL promoted OvCa cell metastasis by regulating EMT and increasing FN1 expression via miR-101 sponging.[25] Moreover, Yokoi *et al*. discovered that when p53 function is lost, FN levels rise, which may alter the biological behavior of ovarian clear cell carcinoma/ ovarian high-grade serous carcinoma. This may have an effect on cell motility and proliferation, and is especially true in tumors with an HNF-1^{+}/$p53^{+}$/$ARID1A^{+}$ immunophenotype. The aberration of p53 can promote EMT/CSC characteristics and inhibit apoptosis,[26] both of which can contribute to the development and maintenance of tumor phenotypic traits.

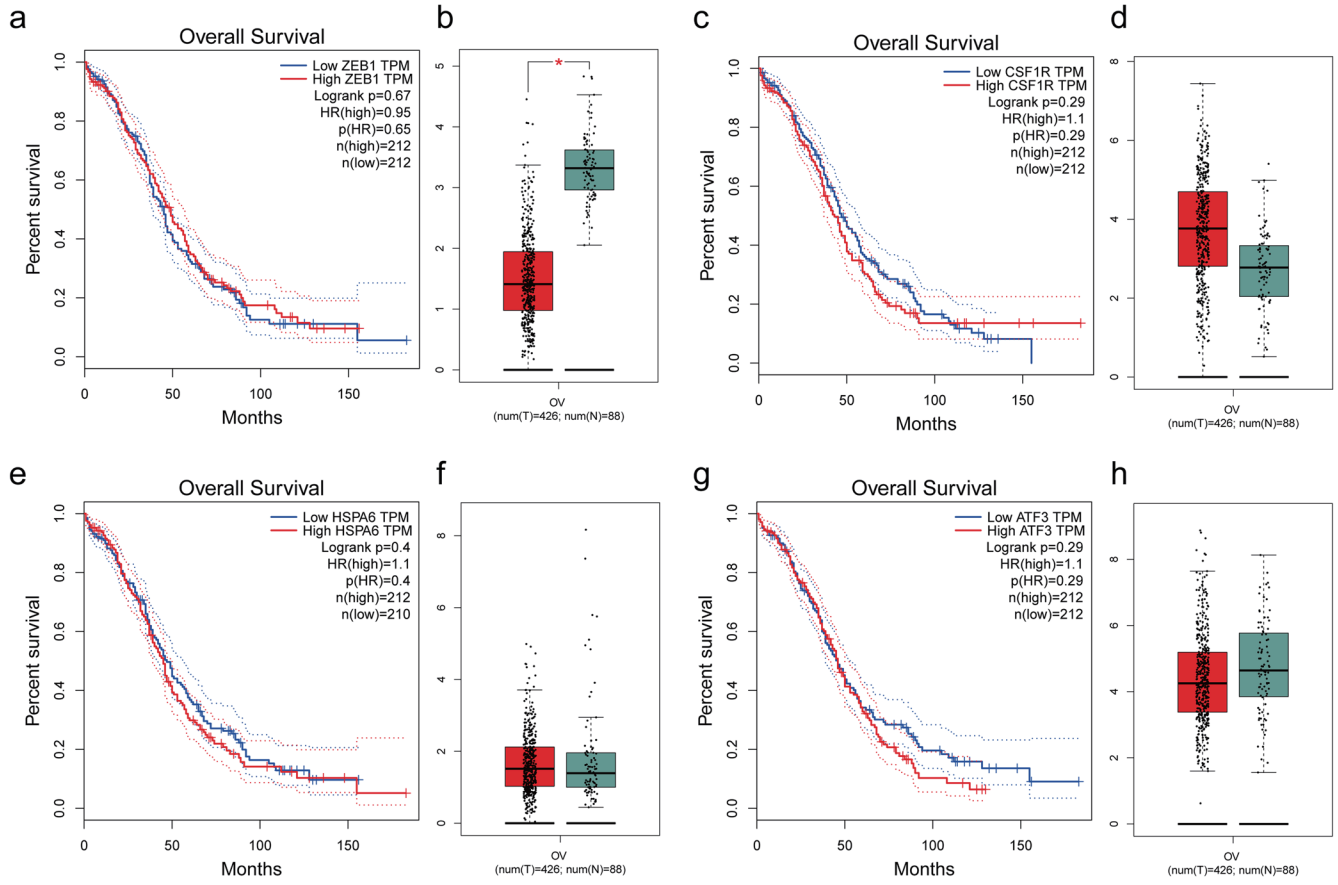

Fig. 4. The overall survival and expression patterns of candidate proteins in the clinical data. We evaluated the important proteins for confirmation in the samples of ovarian cancer patients in clinical databases in terms of gene expression and survival. On average, these proteins had a 60% effect on the mortality of ovarian cancer patients. (a–b) ZEB1; (c–d) CSF1R; (e–f) HSPA6; and (g–h) ATF3.

The peritoneum, a thin membrane coated by mesothelial cells, is a common site for the spread of OvCa. Cell-to-cell interaction or phenotypic changes may arise between OvCa cells and mesothelial cells, since they share the same peritoneal metastatic environment. OvCa cells acquiring resistance to platinum is one such instance. In this research, Yoshihara *et al*. describe how direct cell-to-cell interactions between OvCa and OvCa-associated mesothelial cells (OCAMs) can develop platinum-resistance in OvCa cells. Using *in-vitro* coculturing experimental models and *in-silico* omics data analysis, this group of researchers looked into the mutually beneficial interactions between OvCa cells and human primary mesothelial cells. The studies on the role of OCAMs used both *in-vitro* cell cultures and *in-vivo* animal models. Using *in-vitro* studies, the role of transforming growth factor 1 as the primary stimulator of EMT in OCAMs has been established. OvCa cell behavior can also be affected by the presence of OCAMs in the peritoneal metastatic tumor microenvironment. The interaction of OCAMs on the surface of adjacent cells leads to the activation of the FN1/Akt signaling pathway, which in turn reduces platinum sensitivity in OvCa cells. Hematological examination of peritoneal metastases from OvCa revealed FN1 expression in stromal cells thought to be OvCa mesothelial cell progenitors. Additionally, we used an *in-vivo* animal model to show that OvCa cells activated Akt signaling when in contact with the transforming growth factor 1-stimulated peritoneum. Given that the tumor microenvironment facilitates the development of platinum-resistance in OvCa cells by direct cell-to-cell interaction between OvCa cells and OCAMs,[27] our results suggest that this environment may be an effective therapeutic target for halting the spread of OvCa within the peritoneum. There is a lack of evidence for the involvement of other genes and proteins in OvCa metastasis via CSC functions.

Incorrect cell maturation is often believed to be the root cause of cancer. An expanding body of evidence suggests that CSCs, also known as tumor-initiating cells, are a critical subset of tumor cells that drive not only tumor initiation and progression but also therapy resistance and metastasis relapse and, ultimately, cancer progression and progression.[28] miRNAs from the let-7 family were found for the first time in *C. elegans* and have been found to retain their original functions across all major evolutionary branches, from worms to humans. The regulation of cell proliferation, differentiation, and pluripotency, as well as the initiation and development of cancer, are only some of the many cellular processes in which these miRNAs play a role.[29] The effects of hypoxia on colorectal cancer (CRC) cell apoptosis, migration, invasion, and EMT were studied by Quyang *et al*. The transcriptional activity of FOXA2 is directed toward hsa-let-7 g via targeting c14orf28[30] to prevent hypoxia from triggering EMT. This is done to stop emergency medical treatment.

Using bioinformatics and *in-vitro* tests, Shen *et al*. have demonstrated that ONECUT2 is overexpressed in gastric cancer, while

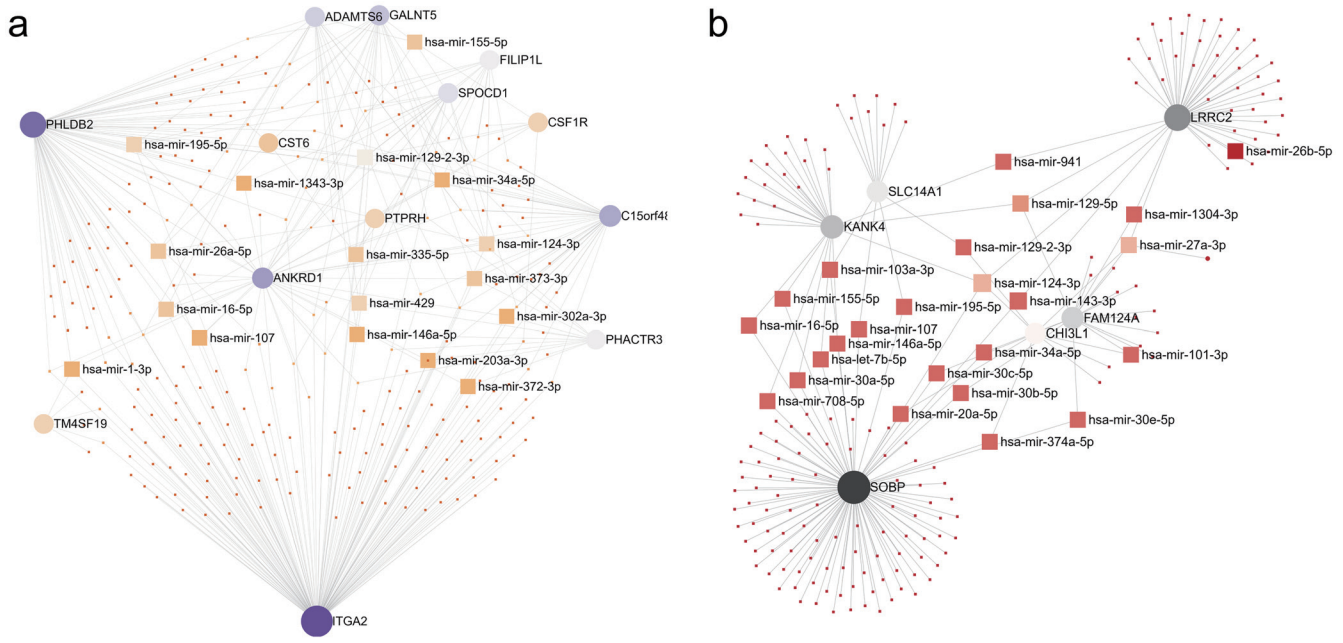

Fig. 5. Investigation of the miRNA and gene target network in ovarian cancer stem cells. Here, the communication network between miRNAs associated with candidate genes and proteins from the previous stage is drawn between high and low expression genes, and important miRNAs involved in the development of ovarian cancer stem cells can be seen. (a) Upregulated miRNAs; (b) downregulated miRNAs.

hsa-miR-15a-5p is downregulated. Immunohistochemical analyses have linked ONECUT2 expression in gastric adenocarcinoma to both disease progression and prognosis, finding that higher levels of expression are associated with fewer differentiated tumors. Gastric cancer stem cells (GCSCs) were isolated from CD133^{+}/CD44^{+} MKN45 by flow cytometry and compared to their parental MKN45 counterparts; GCSCs displayed higher levels of ONECUT2 and lower levels of hsa-miR-15a-5p. These results raise the

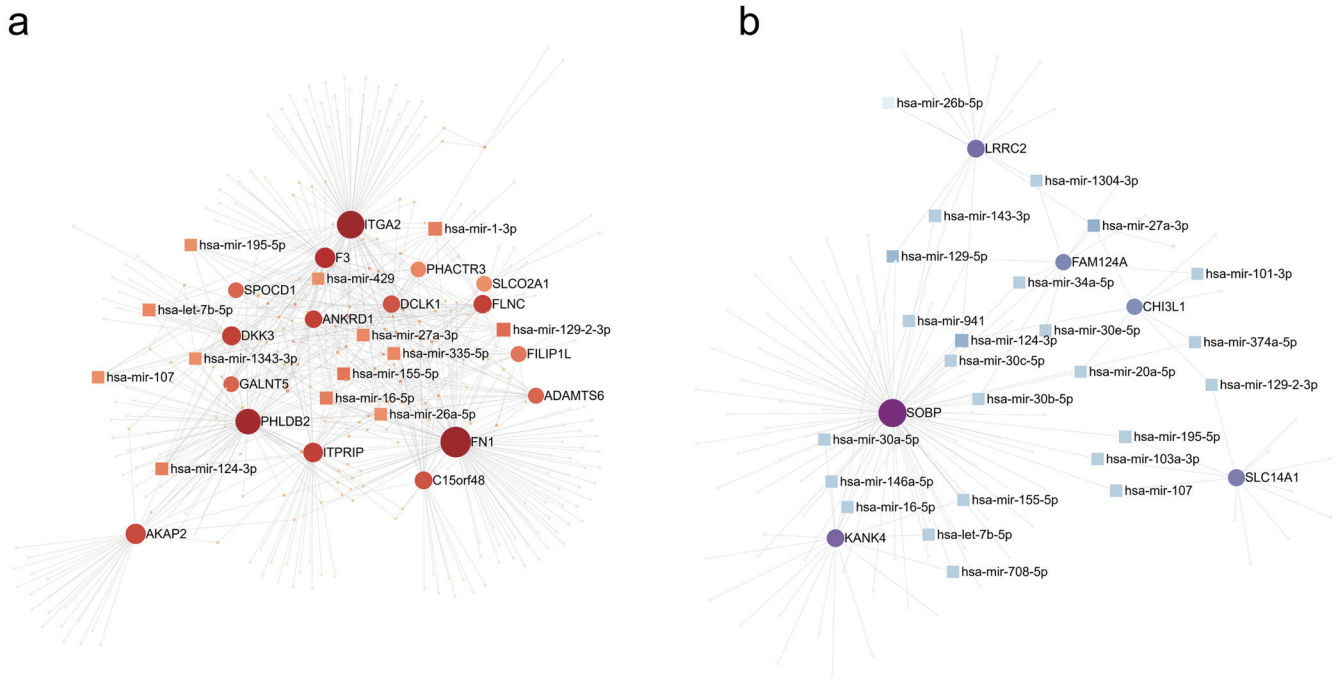

Fig. 6. Selection of hub miRNAs in ovarian cancer stem cells and the extracellular matrix. In order to determine the accuracy of studying and finding important markers in blood, especially miRNAs derived and related to the physiology of cancer stem cells, we checked them in terms of their presence or absence in extracellular vesicles, and we drew their related network. (a) upregulated miRNAs; (b) downregulated miRNAs.

intriguing possibility that GCSCs contribute to the development of gastric cancer. We also conducted *in-vivo* and *ex-vivo* experiments to show that has-miR-15a-5p regulates stemness maintenance, EMT, and chemosensitivity in GCSCs via targeting ONECUT2. The suppression of the G0 phase of GCSCs can be traced back to hsa-miR-15a-5p. It does this via controlling the signaling pathway involving ONECUT2 and β-catenin,[31] which is essential for suppressing the target gene. Although apelin is used as a tumor promoter by a wide range of malignant tumors, the mechanism by which this occurs is not well known. According to previous studies, exosomes have been shown to play a key role in preventing tumor progression and metastasis. For insight into whether apelin promotes lung cancer cell proliferation and invasion, we examined its effect on exosomal miRNA levels. Ran *et al*. overexpressed apelin or a control vector in lung cancer A549 cells using lentiviral transfection. The culture supernatants from each cell type were analyzed to isolate and characterize exosomes. The effects of A-exo and V-exo on the proliferation, apoptosis, colony formation, and invasion of A549 cells were evaluated. We sequenced the miRNAs isolated from A-exo and V-exo using the miRNA-sequencing technique in order to find a potential candidate miRNA. Since A-exo was more readily taken up by A549 cells than V-exo, its effects on A549 cell proliferation, colony formation, migration, and invasion were investigated. V-exo, on the other hand, was taken up less efficiently by A549 cells. The results of exosomal miRNA-sequencing showed that miR-15a-5p was much less abundant in A-exo than in V-exo. miR-15a-5p was also shown to be downregulated in lung cancer tissues and cell lines, suggesting that it may play a role in preventing tumor development. Reduced levels of cell division cycle-associated protein 4 suggested that it might be a target of miR-15a-5p, so Ran *et al*. tested whether or not it could bind to the protein after finding that overexpression of miR-15a-5p in A549 cells was associated with a decrease in cell proliferation, migration, invasion, and the cell cycle. Our data demonstrated that it did not successfully connect with the protein. Inhibiting the exosomal release of miR-15a-5p[32] was found to be the regulatory mechanism by which apelin promotes lung cancer cell proliferation and invasion, which had previously been unknown. miR-15a was downregulated while KCNQ1 opposite strand/antisense transcript 1 (KCNQ1OT1), programmed death-ligand 1 (PD-L1), and cluster of differentiation 8 (CD8) were upregulated in prostate cancer (PC) tissues. When compared to the concentrations in non-PC tissues, this was the finding. miR-15a was able to suppress the expression of PD-L1 by directly binding to the gene's 3′-untranslated region. It was shown that overexpressing miR-15a in PC cells was sufficient to increase the cytotoxicity and proliferation of $CD8^+$ T cells; decrease the apoptosis of $CD8^+$ T cells; and decrease the viability, migration, invasion, and EMT in PC cells while enhancing apoptosis in PC cells. Although PC cells overexpressed miR-15a, this impact still materialized. When overexpression of PD-L1 was discovered, the antitumor effects of miR-15a were rendered null and void. The PD-L1 inhibition that KCNQ1OT1 had been holding onto was lifted when it soaked up miR-15a. By inhibiting $CD8^+$ T-cell cytotoxicity and preventing apoptosis, KCNQ1OT1 played a crucial role in maintaining a variety of malignant states in PC cells. To do this, apoptosis in PC cells was blocked. Overexpression of miR-15a or reduction of KCNQ1OT1 reduced Ras/ERK signaling.[33]

Recently established as a vital regulator in the process of carcinogenesis, circRNAs play a function in the development of CRC. It was unclear, however, how exactly hsa circRNA 002144 contributes to the etiology and progression of CRC. A definite association between hsa circRNA 002144 and a poor prognosis in patients was first proposed after its detection at significantly higher levels in both CRC tissues and cell lines. Both CRC tissues and cell lines have been reported to have this circRNA. Inhibiting hsa circRNA 002144 slowed the development of CRC by decreasing cell viability, proliferation, migration, and invasion while simultaneously increasing apoptosis, as shown by the findings of functional experiments. Knocking down hsa circRNA 002144 inhibited CRC growth and metastasis in *in-vivo* experiments. Increased levels of hsa circRNA 002144, on the other hand, have been linked to a more rapid progression of CRC. In conclusion, hsa circRNA 002144 suppressed miR-615-5p expression and was found to be physically associated with miR-615-5p in the cytoplasm of CRC cells. Furthermore, La ribonucleoprotein 1, translational regulator may also be a target of miR-615-5p. Finally, it was discovered that elevated expression levels of this protein counteract the restraint exerted by low hsa circRNA 002144 levels on CRC development.[34] In addition, Lei *et al*. found that metastatic breast CCs and tissues have the highest concentrations of the miRNA miR-615-3p. Breast cancer cell lines in which miR-615-3p was overexpressed in a stable fashion exhibited enhanced motility *in vitro* and lung metastases *in vivo*. To achieve this, the expression of mesenchymal markers was upregulated, while the expression of epithelial markers was downregulated. Research has shown that downstream signaling of TGF-, type I receptor was enhanced after reintroducing miR-615-3p, which had been shown to target the 3′-untranslated regions of protein interacting with C kinase 1. As a negative feedback regulator, this protein is important in breast CCs. This is achieved by blocking the events required for cell death induction, namely the maturation of pre-miR-615-3p into mature miR-615-3p and the binding of DICER1 to Smad2/3.

The initiation, progression, and acquired resistance of cancer to treatment have all been linked to miRNA dysregulation. Although drug resistance in OvCa cells has been the subject of extensive study, the miRNAs that underlie these phenomena remain unknown. The goal of the study by Diaz *et al*. was to discover miRNAs that might play a role in platinum resistance by comparing the expression of miRNAs in cisplatin-sensitive and -resistant OvCa cells. Several miRNAs were found to be significantly more or less prevalent in each cell line, as determined by the research conducted by Diaz *et al*.[35] As a result, the team reasoned that miRNAs could contribute to platinum resistance. For example, miR-18a-5p (miR-18a), a member of the oncogenic related miR-17-92 cluster, was found to be downregulated in cisplatin-resistant cells. The miR-18a-5p levels were lower in these cells compared to those that were susceptible to cisplatin. The validity of these findings was confirmed by using the real-time polymerase chain reaction. Then, oligonucleotide miRNA mimics (OMMs) were used to raise miR-18a levels, and their effects were studied. Cell growth, proliferation, and invasion were all inhibited when cells were transiently transfected with a miR-18a-OMM as opposed to a negative control OMM. Mice with established OvCa demonstrated significant decreases in the tumor mass and nodule count following intraperitoneal injections of miR-18a-OMM-loaded folate-conjugated liposomes. Patients with OvCa who had greater miR-18a levels than those who had lower miR-18a levels fared better in a survival study using the Kaplan–Meier plotter database. MMP3 was found to be a direct target of the miRNA miR-18a by the use of bioinformatics, real-time polymerase chain reaction, western blotting, and luciferase reporter assays. Increased cell mortality, growth halt, and resistance to invasion were all associated with overexpression of MMP3 in cisplatin-resistant OvCa patients. Based on our findings, targeting miR-18a may be a viable therapeutic method for treating cisplatin-resistant OvCa.[36] We examined head and neck

squamous cell carcinoma (HNSCC) cells for overexpression of miR-18a-5p and downregulation of sorbin and SH3 domain containing 2 (*SORBS2*) using quantitative real-time polymerase chain reaction. miR-18a-5p promoted HNSCC cell proliferation, migration, and invasion, as evidenced by cell counting kit-8, transwell, and flow cytometry assays. To demonstrate that miR-18a-5p binds to *SORBS2*, a dual-luciferase reporter gene assay was performed. Rescue experiments showed that overexpression of miR-18a-5p negatively affected HNSCC cells; however, this effect could be reversed by coercing the cells to express *SORBS2*. Our data as a whole suggest that miR-18a-5p/*SORBS2* is involved in the promotion of malignant phenotypes in HNSCC cells. It is possible that our study's findings could serve as a preclinical standard for HNSCC.[37]

This study's findings, based on a compilation of the available data, support the idea that detecting OvCa at an early stage provides the highest opportunity for successful treatment and survival. The number of survivors could be greatly increased, however, if early detection were part of a widespread screening plan. The dismal 20% five-year survival rate in the fourth stage of the disease is indicative of its extreme hazard. The typical robotized train layout in a hospital's clinical biochemistry facility will necessitate the use of a highly reliable piece of laboratory equipment if it is expected that literally millions of patients of a specific age (over 40 years old?), each of whom needs to be screened once per year. In such labs, this is a common arrangement for chemiluminescent enzyme-linked immunosorbent assay research. Furthermore, several of the most effective procedures for diagnosing OvCa include measurements of a panel of biomarkers as opposed to just one. Therefore, it would be very helpful to have a single piece of machinery that can perform proteome or genomic analysis of all necessary indicators. Proteomic biosensing using a complementary metal-oxide semiconductor device has been proposed,[38] with individual sensors tracking different biomarkers of interest. This would make it possible to assess multiple biomarkers concurrently.

In this case, a reversible flow-injection mode of operation from the sensor is required, as opposed to the fixed mode of operation used in traditional end-point spectroscopic assays. Biosensor technology has great potential in the future, but there are still many obstacles to overcome before it can be widely used in today's clinical biochemistry laboratories. A number of factors have prevented the widespread adoption of devices that measure biomarkers via flow injection, including interference from surface fouling in biological fluid (serum), a lack of specificity to the biomarker being measured, the measurement of the biomarker concentration at anticipated low levels, and sensor stability and reversibility over significant periods of operational time. From a biological standpoint, it would be fantastic if a method could be developed to evaluate a universal marker shared by different OvCa cell lines. This is the best-case scenario, for sure. There are at least five different forms of ovarian epithelial carcinomas that have been identified through research into the disease's pathophysiology, with high-grade serous carcinoma being the most lethal type and accounting for 75% of all cases.

Conclusions

In this study, we investigated and predicted important genes, protein products, and miRNAs in OvCa stem cells. Also, we evaluated miRNAs in terms of their presence and absence in EVs. This approach will aid in the identification of the best markers in the serum or plasma of patients, and related CSCs and will be able to provide diagnosis management and new treatments to prevent the development, metastasis, and recurrence of this cancer.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

All authors participated in study design, data collection and evaluation, drafting of the manuscript, and statistical analysis as well as contributed extensively to the interpretation of the data, conclusions, and figure design. All authors performed editing and approved the final version of this paper for submission. They also participated in the finalization of the manuscript and approved the final draft.

Data sharing statement

All data generated or analyzed during this study are included in this published article.

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