Epigenetic Mechanism and Survival Prognosis Analysis of Serum Exosomes from Ovarian Cancer Patients based on Sequencing Technology and Bioinformatics

Li Xia

School of Basic Medical Sciences, Xinjiang Medical University   https://orcid.org/0000-0001-8812-5788

Huang He (✉ huanghe@csu.edu.cn)

School of Basic Medical Sciences, Xinjiang Medical University   https://orcid.org/0000-0002-4682-8335

Abstract

Backguound: To screen the signaling axis of epigenetic modification in serum exosomes of ovarian cancer patients based on sequencing technology and raw signal analysis, in depth study of the potential mechanism of action of ovarian cancer, prediction of potential therapeutic targets and survival prognosis analysis of potential targets.

Methods: Serum exosomes from three ovarian cancer patients were selected as the experimental group, and serum exosomes from three uterine fibroid patients as the control group, and whole transcriptome of serum exosomes was performed to obtain differentially expressed lncRNA and mRNA in ovarian cancer,The miRcode database and miRNA target gene prediction website were used to predict the target genes, Cytoscape software was used to draw a ceRNA network model of epigenetic modification of ovarian cancer serum exosomes, and the R language was used for GO and KEGG enrichment analysis of the target genes. Finally, the TCGA website was used to download clinical and expression data related to ovarian cancer, and the common potential target genes obtained in the previous period were analyzed for survival

Results: A total of 117 differentially expressed lncRNAs as well as 513 differentially expressed mRNAs (P < 0.05, |log2 FC|≥ 1.0) were obtained by combining sequencing data and raw signal analysis, and 841 predicted target genes were reciprocally mapped by combining mircode database and miRNA target gene prediction website, resulting in 11 potential target genes related to ovarian cancer (FGFR3, BMPR1B, TRIM29, FBN2, PAPPA, CCDC58, IGSF3, FBXO10, GPAM, HOXA10, LHFPL4), and survival prognosis analysis of the above 11 target genes revealed that the survival curve was statistically significant (P < 0.05) for HOXA10 only genes, but not for the other genes, and through enrichment analysis, we found that the above target genes were mainly involved in biological processes such as regulation of transmembrane receptor protein kinase activity, structural molecule activity with elasticity, transforming growth factor - activated receptor activity, and GABA receptor binding, and were mainly enriched in signaling pathways regulating stem cell pluripotency, bladder cancer, glycerolipid metabolism, central carbon metabolism of cancer, tyrosine stimulation to EGFR in signaling pathways such as resistance to enzyme inhibitors.

Conclusions: The serum exosomal DIO3OS-hsa-miR-27a-3p-HOXA10 epigenetic modification signaling axis affects ovarian cancer development and disease survival prognosis by targeting transcriptional dysregulation pathways in cancer.

Introduction

Ovarian cancer is one of the three major gynecological malignancies that seriously affect women's health, second only to incidence of 5.0/100000, mortality of 3.1/100000, and morbidity is third only to cervix and uterine corpus cancer(Siegel, Miller, & Jemal, 2020). Although the surgery and chemotherapy of ovarian cancer have made great progress in recent years, the incidence and case fatality rate are still

increasing year by year (Rocconi et al., 2020). due to its insidious onset, about 70% of patients have advanced disease at the time of discovery, and its malignancy is high and the prognosis is poor. The 5-year survival rate of ovarian cancer patients is only 47% (Kandalaft, Odunsi, & Coukos, 2020). Current treatments for ovarian cancer mainly apply tumor cytoreductive surgery and chemotherapy drugs platinum, paclitaxel, and other treatments (Onda et al., 2020). The complete response rate of this standard treatment regimen in advanced ovarian cancer can reach 40% - 60%,But more than 90% of patients who relapse after 8 months and develop resistance to chemotherapeutic drugs, eventually succumb to ovarian cancer (Coleman et al., 2019). thus, the prognosis of ovarian cancer remains to be further improved, and it is very necessary to explore novel therapeutic targets and means. Epigenetics is the premise of studying heritable changes in gene expression and function that do not involve DNA sequence alterations through certain mechanisms, mainly including regulatory mechanisms such as DNA methylation, histone modification, and RNA editing (Tran, MacFarlane, Kong, O'Connor, & Yu, 2016).As research has progressed, it has been found that epigenetic inheritance plays an important role in the development of a variety of major diseases. In ovarian cancer, endometrial cancer, as well as cervical cancer, there have been many studies demonstrating the influence of genetic and epigenetic modifications on tumor initiation and progression(Efthymia Papakonstantinoua et al., 2020; Oliveira et al., 2021; Xie et al., 2021). LncRNAs are a class of RNAs with transcripts longer than 200nt that play important regulatory roles in regulating gene expression, life development and disease development, and mainly in the nucleus to regulate epigenetic modifications (Hosono et al., 2017).In this study, we aimed to explore the signaling axis of epigenetic modification in serum exosomes from ovarian cancer patients and their potential therapeutic targets using whole transcriptome sequencing, TEM, and Sanger analysis.

Materials And Methods

Information and source The serum of ovarian cancer and uterine fibroid patients in our hospital was collected, exosomes were extracted, and whole transcriptome expression differences were detected by sequencing, in which the experimental group was ovarian cancer patients (the samples were preoperative serum of ovarian cancer patients), postoperative histopathological results were malignant, and the control group was uterine fibroid patients (the samples were preoperative serum), no previous ovarian disease, postoperative pathological results showed normal inclusion criteria: (1) those who met the diagnostic criteria of ovarian cancer (Lokshin, 2012);(2) Those with a telephone follow-up were eligible. Exclusion criteria were (1) those with severe cardiac, hepatic, and renal dysfunction, (2) those with other malignant tumors or systemic infectious diseases, (3) those with other gynecological diseases, (4) who withdrew from the investigator halfway, and (5) those with incomplete clinical data.

Methods

1 Extraction of serum exosomes by ultracentrifugation The serum was thawed in medium at 37 ∘ C for 30 min and centrifuged at 2000 × g, 4 ° C, and the supernatant was removed to a new centrifuge tube and centrifuged again at 10000 × g, 4 ° C, and 45 min to remove larger vesicles. The supernatant was

extracted and filtered through a 0.45 μ M filter membrane and the filter was collected. The filter was removed to a new centrifuge tube and the ultrarotor was selected at 4 ∘ C, 100000× g for 70 min. to remove the supernatant, resuspend in 10 ml prechilled 1 × PBS, select the ultrarotor, and centrifuge again at 4 ° C, 100000 × g, and ultracentrifuge for 70 min. to remove the supernatant, resuspend in 100 μ L prechilled 1 × PBS, take 20 μ L for identification under electron microscopy, and store the remaining exosomes at - 80 ° C.

2 Transmission electron microscope observation The exosomes were taken out 10 μ L, pipetting the sample 10 μ l dropwise added onto the copper grid to precipitate for 1 min, filter paper pipetted off the floating liquid. Uranium acetate 10 μ l dropwise added onto the copper grid to precipitate for 1 min, filter paper pipetted off the floating liquid. Drying at room temperature for several minutes at 100 kV for electron microscope detection imaging (Rikkert, Nieuwland, Terstappen, & Coumans, 2019). To obtain the transmission electron microscope imaging results.

3 Differential expression gene screening The sequencing data were background corrected, normalized and expression values calculated using the Bioconductor R package in R, and the limma package in R was used to calculate the differential expression lncRNAs and mRNAs between the two groups, setting P < 0.05, and the magnitude of the expression change ≥ twofold (|log2 FC| ≥ 1.0).To screen the criteria of differential genes, in which log2 FC ≥ 1.0 represents upregulated lncRNA and mRNA expression, and log2FC≤ - 1.0 represents downregulated lncRNA and mRNA expression, respectively. Finally, the differential expression lncRNAs and mRNAs, that is, differentially expressed lncRNAs (DElncRNAs) as well as differentially expressed genes in ovarian cancer as well as uterine leiomyoma control group were obtained(Differentially Expressed Genes, DEGs). Heatmap plotting as well as cluster analysis of the screened DElncRNAs, DEGs were performed using the Heatmap package, and the P values in the differentially processed data were -log10 transformed and -log10 (P values) were grouped according to log2 FC(upregulated lncRNAs group, downregulated lncRNAs group, lncRNAs group without statistical significance as well as upregulated DEGs group, downregulated DEGs group, DEGs group without statistical significance), the post-treatment data were imported into GraphPad Prism 8 to draw volcano plots.

4 miRNAs predicted to be bound by lncRN As The sequencing datasets were differentially analyzed using the miRcode database the resulting delncrnas were predicted to be bound by miRNAs to further explore the underlying pathogenesis of the disease the differentially analyzed processed data were compared with the mircode database for the upregulated and downregulated DElncRNAs according to the set filtering criteria(http://www.mircode.org)among the 'Highly conserved microRNA families' dataset was comparatively analyzed to derive miRNAs with potential binding to DElncRNAs.

5 Prediction of miRNA target genes Using the miRNAs derived from the previous step that were potentially bound by DElncRNAs, miRNA target gene prediction was performed. The miRNAs potentially bound to the differentially expressed lncRNAs were separately input into online miRNA target gene prediction websites miRDB, miRTarBase, TargetScan for target gene prediction, and the resulting predicted target genes were

aligned and mapped with the differentially expressed gene DEGs obtained from sequencing, and the differentially expressed lncRNAs - miRNA - mRNAs were further obtained by Cytoscape 3.7.2 of the ceRNA interaction network model (Shannon et al., 2003).

6 GO and KEGG enrichment analysis The common potential target genes predicted from the previous step were entered into the DAVID database to select species as human (Homo spaiens) for gene ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) signaling pathway analysis(Huang da, Sherman, & Lempicki, 2009a, 2009b).Among these, GO analysis mainly included the cellular component (CC), molecular function (MF) of the differential genes ,and biological process (BP). Target genes were screened at P < 0.05 to analyze the biological processes of potential target genes and major signaling pathways. Pathway diagrams were established using Bioconductor-pathview in R software (version R x64 3.5.1).

7 Survival prognosis analysis of potential target genes The resulting common potential target genes from 1.2.5 were subjected to prognostic survival analysis using TCGA(https://portal.gdc.cancer.gov/)clinical and expression of Humans; R software was utilized to collate summary summary clinical data, and further derive its expression matrix. Finally, the survival package was utilized to perform survival analysis on the common potential target genes obtained in the previous period.

Results

Characteristics of exosomes in serum

To identify whether the particles isolated from serum were indeed exosomes, vesicles were identified by transmission electron microscopy (TEM), size quality assessment, and protein quality assessment. TEM images showed that serum exosomes were morphologically intact, spherical, and uniform in size, with diameters ranging from 30 to 200 nm, corresponding to the conventional size range of exosomes (Figure1).As expected, the results of protein quantity assessment showed that the commonly used exosomal markers such as CD9, CD63, CD81, and TSG101 were abundantly expressed in the isolated pellets, and all the above results showed the main characteristics of exosomes, which confirmed the successful isolation of exosomes from serum samples.

Screening of differentially expressed genes

After the setting of screening conditions, our study was derived from 3 preoperative serum samples of ovarian cancer patients (aged 39.0 ± 6.0 years) and 3 serum samples of uterine fibroid patients (aged 58.0 ± 13.0 years) collected in our hospital, and the baseline characteristics of the samples are shown in Table 1. The sequencing data were background corrected, normalized, and normalized using the R package, and the PCA of the corrected data distribution is shown in Figure2. According to the P values(P) < 0.05, and those with ≥ twofold change in expression ($|\log_2 FC| \geq 1.0$)to select the criteria of differentially expressed long noncoding RNAs and coding RNAs, 117 differentially expressed lncRNAs,

including 36 upregulated lncRNAs and 81 downregulated lncRNAs, and 513 differentially expressed mRNAs, including 231 upregulated mRNAs and 282 downregulated mRNAs, were selected in the sequencing data according to the p-value(P) the top 50 most significant differentially expressed long non coding RNAs and coding RNAs were screened and plotted as a Heatmap, see Figure 3. Where red represents upregulation of gene expression and green represents downregulation of gene expression. The p value in the sequencing data after differential analysis was - log10 transformed and - log10 (P value) was grouped according to $\log_2$ FC(upregulated lncRNAs group, downregulated lncRNAs group, lncRNAs group without statistical difference as well as upregulated DEGs group, downregulated DEGs group, DEGs group without statistical significance), the post-treatment data were imported into GraphPad Prism 8 to draw a volcano plot, see Figure 4.

Table 1
Table of sample baseline characteristics

Sample size	age	Tumor Stage			sample
	(year, $\overline{X}$ ±SD)	I	II	III	
hysteromyoma (n=3)	39.0 ± 6.0	-	-	-	Serum exosomes
Ovarian cancer (n=3)	58.0 ±13.0	IC(1)	-	IIIC(2)	Serum exosomes

Prediction of lncRNA bound miRNAs

Differential analysis of sequencing data using the miRcode database was used to obtain differentially expressed lncRNAs for prediction of miRNAs with which to bind, to further explore the underlying pathogenesis of the disease. The differentially analyzed processed data were compared with the miRcode database according to the set filtering criteria for differential lncRNAs the up - and down regulated DElncRNAs were respectively (http://www.mircode.org/)among the 'highly conserved microRNA families' dataset was comparatively analyzed to obtain the miRNAs potentially bound to the differentially expressed lncRNAs, and the results were shown in Table2.

Table 2
Partial miRNAs potentially bound by differentially expressed lncRNAs

lncRNA	miRNA
C10orf95	hsa-miR-503、hsa-miR-7、hsa-miR-7ab、hsa-miR-143、hsa-miR-1721、hsa-miR-4770、hsa-miR-150...
LINC00358	hsa-miR-141、hsa-miR-200a、hsa-miR-150、hsa-miR-5127、hsa-miR-1ab、hsa-miR-206...
FAM215B	hsa-miR-503、hsa-miR-139-5p、hsa-miR-205、hsa-miR-205ab、hsa-miR-217、hsa-miR-218...
EGOT	hsa-miR-135ab、hsa-miR-135a-5p、hsa-miR-141、hsa-miR-200a、hsa-miR-143、hsa-miR-1721...
CRNDE	hsa-miR-9、hsa-miR-9ab、hsa-miR-135ab、hsa-miR-135a-5p、hsa-miR-140、hsa-miR-140-5p...
...	...

CeRNA network construction

Using the miRNAs potentially bound to the differentially expressed lncRNAs from the previous step, miRNA target gene prediction was performed and aligned with the DEGs derived from the previous differential analysis, see Figure 5. A to further explore the underlying pathogenesis of the disease. The miRNAs potentially bound to the differentially expressed lncRNAs will be input into online miRNA target gene prediction websites miRDB, miRTarBase, TargetScan for target predictionGene prediction, the results are shown in Table 3, and further through Cytoscape 3.7.2 software to get the CeRNA interaction network model of differentially expressed lncRNAs - miRNA - mRNA, see Figure 5. B.

Table 3
miRNA target gene prediction

miRNA	Gene	miRDB	miRTarBase	TargetScan	Sum
hsa-miR-129-5p	SORBS2	1	1	1	3
hsa-miR-125b-5p	PPAT	1	1	1	3
hsa-miR-23b-3p	PTK2B	1	1	1	3
hsa-miR-129-5p	RSBN1	1	1	1	3
hsa-miR-135a-5p	STAT6	1	1	1	3
hsa-miR-24-3p	PER2	1	1	1	3
hsa-miR-1297	FRAT2	1	1	1	3
hsa-miR-10a-5p	CHL1	1	1	1	3
hsa-miR-107	LATS2	1	1	1	3
hsa-miR-24-3p	AVL9	1	1	1	3
...	...	-	-	-	-

Target gene survival prognosis analysis

Utilizing TCGA(https://portal.gdc.cancer.gov/)the clinical and transcriptomic expression data related to ovarian cancer were downloaded, 379 related datasets were generated according to the screening conditions set in the previous period, the pooled summary clinical data were collated using R software, and the expression matrix was further derived, and finally, the common potential target genes obtained in the previous period were subjected to survival analysis using the survival package, which revealed that the survival curve of HOXA10 only gene had a statisticalThe statistical significance (P < 0.05) was considered, but none of the other indexes showed significant statistical significance, see Figure.6.

GO and KEGG pathway enrichment analysis of target genes

GO and KEGG pathway enrichment analyses of potential target genes were performed using the DAVID database, respectively, and finally the go functions of the core genes mainly involved in transmembrane receptor protein kinase activity, structural molecule activity with elasticity, transforming growth factor - β activated receptor activity, and GABA receptor binding, as shown in Figure 7. Results of KEGG pathway enrichment analysis showed that KEGG of potential target genesThe pathways are mainly involved in: signaling pathways regulating stem cell pluripotency, bladder cancer, glycerolipid metabolism, central carbon metabolism in cancer, resistance to EGFR tyrosine kinase inhibitors, etc. see Figure 7. B. schematic diagram of signaling pathways related to HOXA10 gene, see Figure 7. C.

Disscussion

Epigenetics is the premise of studying heritable changes in gene expression and function that arise through certain mechanisms that do not involve DNA sequence alterations, including primarily regulatory mechanisms such as DNA methylation, histone modifications, and RNA editing (Portela & Esteller, 2010).As research has progressed, it has been found that epigenetic inheritance plays an important role in the development of a variety of major diseases(Navarro et al., 2014; Sarkargar, Mazaheri, Zare, & Hajihosseini, 2021; Szukiewicz et al., 2021). A number of studies have demonstrated the influence of genetic and epigenetic modifications on tumor initiation and progression in ovarian cancer, endometrial cancer, as well as cervical cancerIn contrast to gene mutations, epigenetics does not act by altering the genomic sequence, but by methylating modifications, histone modifications, miRNA regulation, etc. aberrant methylation, histone modification errors, or miRNA dysregulation are closely associated with tumor cell proliferation, autophagy, apoptosis, cell-cell adhesion, invasion, and metastasis (Herceg & Vaissiere, 2011). therefore, this study aimed to mine the differentially expressed genes in ovarian cancer patients with the help of whole transcriptome sequencing technology and Sanger analysis, in order to explore novel therapeutic targets and diagnostic means.

According to statistics, gene mutations account for up to 1 / 4 of ovarian cancer cases. Currently, BRCA1 and BRCA2 have been found to be susceptibility genes for ovarian cancer (Wu et al., 2017), and additional BRIP1, RAD51C, rad51D and mismatch repair genes also play a role (Suszynska, Ratajska, & Kozlowski, 2020). LncRNA HAND2-AS1 / miR-340-5p / BCL2L11 axis can promote proliferation and apoptosis of ovarian cancer through CeRNA mechanism and affect patient survival prognosis(Chen et al., 2019) .In addition, lncRNA MALAT can reach human umbilical vein endothelial cells in a paracrine manner with the help of exosomes from the serum of ovarian cancer patients to regulate angiogenesis by regulating the expression of angiogenesis related genes (Qiu et al., 2018). Finally, lncRNA LINC00161 / miR-128 / MAPK pathway can promote the development of platinum resistance in ovarian cancer tissues (Xu, Zhou, Wu, Wang, & Lu, 2019).In conclusion, our results demonstrated that aberrant expression of lncRNAs could affect several processes, such as tumor proliferation, invasion, metastasis, epithelial mesenchymal transition, vascularization, and platinum chemoresistance, Regulation of ovarian cancer occurrence and development. Studies on lncRNA mechanism of action in ovarian cancer have mainly focused on the mechanism of epigenetic modification: lncRNAs and miRNAs interact with each other, lncRNAs can act as adsorption sponges for miRNAs, and the changes in their expression can lead to changes in miRNA expression, which in turn causes abnormal expression of mRNAs. In addition, lncRNAs also have an interaction relationship with serum exosomes and so on, so this study through extracting serum exosomes, identifying the differentially expressed genes in ovarian patients, deeply investigating the interrelationships between lncrnas and miRNAs, mRNAs and exosomes, and actively searching for specific serum biological markers in ovarian cancer patients to improve the early diagnosis rate of ovarian cancer.

In this study, we performed whole transcriptome sequencing to discover 117 differentially expressed lncRNAs as well as 513 differentially expressed mRNAs by extracting serum exosomes from ovarian cancer patients, and combined 841 predicted target genes derived from miRcode database and miRNA target gene prediction website to map with each other to obtain 11 potential target genes related to ovarian cancer(FGFR3、BMPR1B、TRIM29、FBN2、PAPPA、CCDC58、IGSF3、FBXO10、GPAM、HOXA10、LHFPL4)Moreover, GO/ KEGG enrichment analysis of the above 11 target genes revealed that the above targets were mainly involved in regulating biological processes such as transmembrane receptor protein kinase activity, structural molecule activity with elasticity, transforming growth factor - activated receptor activity, and GABA receptor binding, and were mainly enriched in signaling pathways regulating stem cell pluripotency, bladder cancer, glycerolipid metabolism, and cancer Hub carbon metabolism, resistance to EGFR tyrosine kinase inhibitors) and other signaling pathways. Finally, survival prognosis analysis of the above targets identified a statistically significant ($P < 0.05$) survival curve only for the HOXA10 gene, and HOXA10 gene is mainly involved in the DIO3OS-hsa-miR-27a-3p-HOXA10 epigenetic modification signaling axis to affect the occurrence and development of ovarian cancer and the prognosis change of disease survival in this study.

The long noncoding RNA DIO3OS has been implicated in the development and progression of a variety of tumors(Cui et al., 2019; M. Wang et al., 2021; Z. Wang, Song, Ye, & Li, 2020), however, its specific role in the development and progression of ovarian cancer has not been investigated. In addition, hsa-miR-27a-3p has been shown to affect tumor proliferation, invasion, metastasis in glioblastoma, intrahepatic cholangiocarcinoma, and other malignancies(Salmani et al., 2021; Weiyu Xu, 2020) .In parallel, upregulated expression of HOXA10 promotes epithelial mesenchymal transition as well as proliferation, migration and invasion of ovarian cancer cells and decreases patient survival (Jiang et al., 2014; Liu et al., 2018; Nie et al., 2021).Therefore, the target gene HOXA10 may affect the prognosis of patients with ovarian cancer by regulating transcriptional dysregulation pathways in cancer, while affecting processes such as tumor proliferation, invasion, metastasis, epithelial mesenchymal transition, vascularization, and platinum chemoresistance.

Conclusion

In conclusion, the serum exosomal DIO3OS-hsa-miR-27a-3p-HOXA10 epigenetic modification signaling axis affects ovarian cancer development and disease survival prognosis by targeting transcriptional dysregulation pathways in cancer.

Declarations

Acknowledgements

Authors' contributions

The authors contributed to this study and manuscript in the following manner:data collection,statistical analysis,writing and editing,supervision,funding acquisition,XL; Guidance and review,HH.All authors read and approved the final manuscript.

Availability of data and materials

The data used to support the study are included in the article.

Declarations

Ethics approval and consent to participate

The ethical issues involved in this paper have been passed by the ethics review committee of Xinjiang Medical University when applying for the subject (isolation, identification, morphology and proteomics of ovarian cancer cell exosomes, Project No. 2018d01c273).

Competing interests

The authors declare that they have no competing interests.

References

1. Chen J, Lin Y, Jia Y, Xu T, Wu F, Jin Y. LncRNA HAND2-AS1 exerts anti-oncogenic effects on ovarian cancer via restoration of BCL2L11 as a sponge of microRNA-340-5p. J Cell Physiol. 2019;234(12):23421–36. doi:10.1002/jcp.28911.

2. Coleman RL, Fleming GF, Brady MF, Swisher EM, Steffensen KD, Friedlander M,.. . Bookman MA. Veliparib with First-Line Chemotherapy and as Maintenance Therapy in Ovarian Cancer. N Engl J Med. 2019;381(25):2403–15. doi:10.1056/NEJMoa1909707.

3. Cui K, Jin S, Du Y, Yu J, Feng H, Fan Q, Ma W. Long noncoding RNA DIO3OS interacts with miR-122 to promote proliferation and invasion of pancreatic cancer cells through upregulating ALDOA. Cancer Cell Int. 2019;19:202. doi:10.1186/s12935-019-0922-y.

4. Efthymia Papakonstantinoua G, Androutsopoulosa S, Logothetib G, Adonakisa I, Maroulisc, Tzelepib V. (2020). DNA Methylation in Epithelial Ovarian Cancer_ Current Data and Future Perspectives. *Current molecular pharmacology*.

5. Herceg Z, Vaissiere T. Epigenetic mechanisms and cancer: an interface between the environment and the genome. Epigenetics. 2011;6(7):804–19. doi:10.4161/epi.6.7.16262.

6. Hosono Y, Niknafs YS, Prensner JR, Iyer MK, Dhanasekaran SM, Mehra R,.. . Chinnaiyan AM. Oncogenic Role of THOR, a Conserved Cancer/Testis Long Non-coding RNA. Cell. 2017;171(7):1559–72 e1520. doi:10.1016/j.cell.2017.11.040.

7. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009a;37(1):1–13. doi:10.1093/nar/gkn923.

8. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009b;4(1):44–57. doi:10.1038/nprot.2008.211.

9. Jiang Y, Chu Y, Tang W, Wan Y, Zhang L, Cheng W. Transcription Factor WT1 and Promoter CpG Hypomethylation Coactivate HOXA10 Expression in Ovarian Cancer. Curr Pharm Des. 2014;20:1647–54.

10. Kandalaft LE, Odunsi K, Coukos G. Immune Therapy Opportunities in Ovarian Cancer. Am Soc Clin Oncol Educ Book. 2020;40:1–13. doi:10.1200/EDBK_280539.

11. Liu J, Jiang Y, Wan Y, Zhou S, Thapa S, Cheng W. MicroRNA665 suppresses the growth and migration of ovarian cancer cells by targeting HOXA10. Mol Med Rep. 2018;18(3):2661–8. doi:10.3892/mmr.2018.9252.

12. Lokshin AE. The quest for ovarian cancer screening biomarkers: are we on the right road? Int J Gynecol Cancer. 2012;22 *Suppl*(1):35–40. doi:10.1097/IGC.0b013e318251ccaa.

13. Navarro A, Yin P, Ono M, Monsivais D, Moravek MB, Coon JS, t.,.. . Bulun SE. 5-Hydroxymethylcytosine promotes proliferation of human uterine leiomyoma: a biological link to a new epigenetic modification in benign tumors. J Clin Endocrinol Metab. 2014;99(11):E2437–45. doi:10.1210/jc.2014-2264.

14. Nie S, Zhang L, Liu J, Wan Y, Jiang Y, Yang J,.. . Cheng W. ALKBH5-HOXA10 loop-mediated JAK2 m6A demethylation and cisplatin resistance in epithelial ovarian cancer. J Exp Clin Cancer Res. 2021;40(1):284. doi:10.1186/s13046-021-02088-1.

15. Oliveira D, Hentze J, O'Rourke CJ, Andersen JB, Hogdall C, Hogdall EV. DNA Methylation in Ovarian Tumors-a Comparison Between Fresh Tissue and FFPE Samples. Reprod Sci. 2021. doi:10.1007/s43032-021-00589-0.

16. Onda T, Satoh T, Ogawa G, Saito T, Kasamatsu T, Nakanishi T,.. . Japan Clinical Oncology G. Comparison of survival between primary debulking surgery and neoadjuvant chemotherapy for stage III/IV ovarian, tubal and peritoneal cancers in phase III randomised trial. Eur J Cancer. 2020;130:114–25. doi:10.1016/j.ejca.2020.02.020.

17. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol. 2010;28(10):1057–68. doi:10.1038/nbt.1685.

18. Qiu JJ, Lin XJ, Tang XY, Zheng TT, Lin YY, Hua KQ. Exosomal MetastasisAssociated Lung Adenocarcinoma Transcript 1 Promotes Angiogenesis and Predicts Poor Prognosis in Epithelial Ovarian Cancer. Int J Biol Sci. 2018;14(14):1960–73. doi:10.7150/ijbs.28048.

19. Rikkert LG, Nieuwland R, Terstappen L, Coumans FAW. Quality of extracellular vesicle images by transmission electron microscopy is operator and protocol dependent. J Extracell Vesicles. 2019;8(1):1555419. doi:10.1080/20013078.2018.1555419.

20. Rocconi RP, Grosen EA, Ghamande SA, Chan JK, Barve MA, Oh J,.. . Coleman RL. Gemogenovatucel-T (Vigil) immunotherapy as maintenance in frontline stage III/IV ovarian cancer (VITAL): a randomised, double-blind, placebo-controlled, phase 2b trial. The Lancet Oncology. 2020;21(12):1661–72. doi:10.1016/s1470-2045(20)30533-7.

21. Salmani T, Ghaderian SMH, Hajiesmaeili M, Rezaeimirghaed O, Hoseini MS, Rakhshan A,.. . Akbarzadeh R. Hsa-miR-27a-3p and epidermal growth factor receptor expression analysis in glioblastoma FFPE samples. Asia Pac J Clin Oncol. 2021;17(5):e185–90. doi:10.1111/ajco.13399.

22. Sarkargar F, Mazaheri M, Zare A, Hajihosseini R. (2021). Investigation of epigenetic modifier on HDAC1 and microRNA-410 expression in ovarian cancer cell lines. Gene Reports, 24. doi:10.1016/j.genrep.2021.101240.

23. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D,.. . Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504. doi:10.1101/gr.1239303.

24. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30. doi:10.3322/caac.21590.

25. Suszynska M, Ratajska M, Kozlowski P. BRIP1, RAD51C, and RAD51D mutations are associated with high susceptibility to ovarian cancer: mutation prevalence and precise risk estimates based on a pooled analysis of ~30,000 cases. J Ovarian Res. 2020;13(1):50. doi:10.1186/s13048-020-00654-3.

26. Szukiewicz D, Stangret A, Ruiz-Ruiz C, Olivares EG, Soritau O, Susman S, Szewczyk G. Estrogen- and Progesterone (P4)-Mediated Epigenetic Modifications of Endometrial Stromal Cells (EnSCs) and/or Mesenchymal Stem/Stromal Cells (MSCs) in the Etiopathogenesis of Endometriosis. Stem Cell Rev Rep. 2021;17(4):1174–93. doi:10.1007/s12015-020-10115-5.

27. Tran TK, MacFarlane GR, Kong RY, O'Connor WA, Yu RM. Potential mechanisms underlying estrogen-induced expression of the molluscan estrogen receptor (ER) gene. Aquat Toxicol. 2016;179:82–94. doi:10.1016/j.aquatox.2016.08.015.

28. Wang M, Li J, Zuo Z, Ren C, Tang T, Long C.. . Huang J. Long non-coding RNA DIO3OS/let-7d/NF-kappaB2 axis regulates cells proliferation and metastasis of thyroid cancer cells. J Cell Commun Signal. 2021;15(2):237–50. doi:10.1007/s12079-020-00589-w.

29. Wang Z, Song L, Ye Y, Li W. Long Noncoding RNA DIO3OS Hinders Cell Malignant Behaviors of Hepatocellular Carcinoma Cells Through the microRNA-328/Hhip Axis. Cancer Manag Res. 2020;12:3903–14. doi:10.2147/CMAR.S245990.

30. Weiyu Xu SY, Jianping Xiong3, Junyu Long,Yongchang Zheng, Xinting Sang. (2020). CeRNA regulatory network-based analysis to study the roles of noncoding RNAs in the pathogenesis of intrahepatic cholangiocellular carcinoma. Aging (Albany NY), 12(2), 1047-1086.

31. Wu X, Wu L, Kong B, Liu J, Yin R, Wen H,.. . Liu Y. The First Nationwide Multicenter Prevalence Study of Germline BRCA1 and BRCA2 Mutations in Chinese Ovarian Cancer Patients. Int J Gynecol Cancer. 2017;27(8):1650−7. doi:10.1097/IGC.0000000000001065.

32. Xie W, Sun H, Li X, Lin F, Wang Z, Wang X. Ovarian cancer: epigenetics, drug resistance, and progression. Cancer Cell Int. 2021;21(1):434. doi:10.1186/s12935-021-02136-y.

33. Xu M, Zhou K, Wu Y, Wang L, Lu S. Linc00161 regulated the drug resistance of ovarian cancer by sponging microRNA-128 and modulating MAPK1. Mol Carcinog. 2019;58(4):577−87. doi:10.1002/mc.22952.

Figures
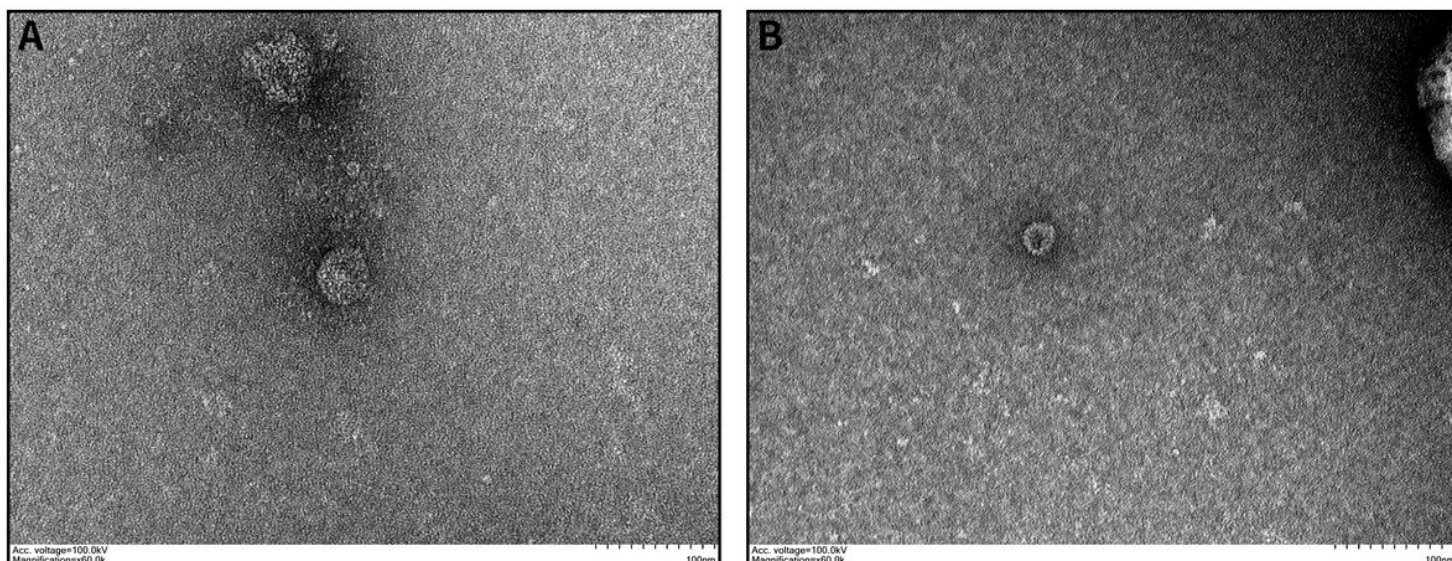
Figure 1

Characteristics of exosomes isolated from serum samples:A. B. serum derived exosome morphology visualized by TEM, indicating that the diameter of the isolated exosomes was in the range of 30-200 nm.
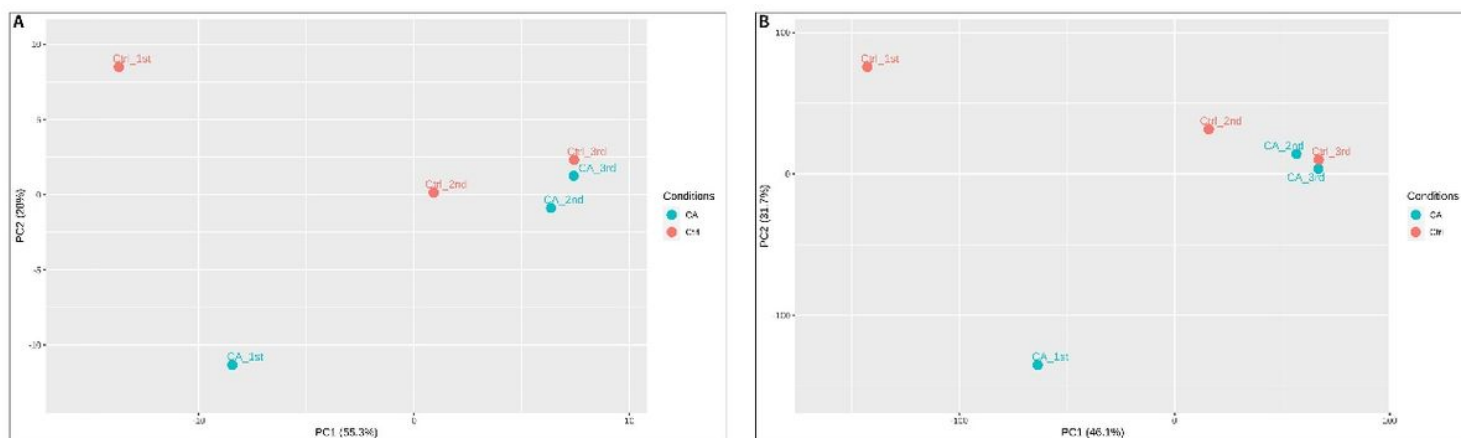
Figure 2

PCA distribution of ovarian cancer sequencing data:A lncRNA PCA distribution. B mRNA PCA distribution

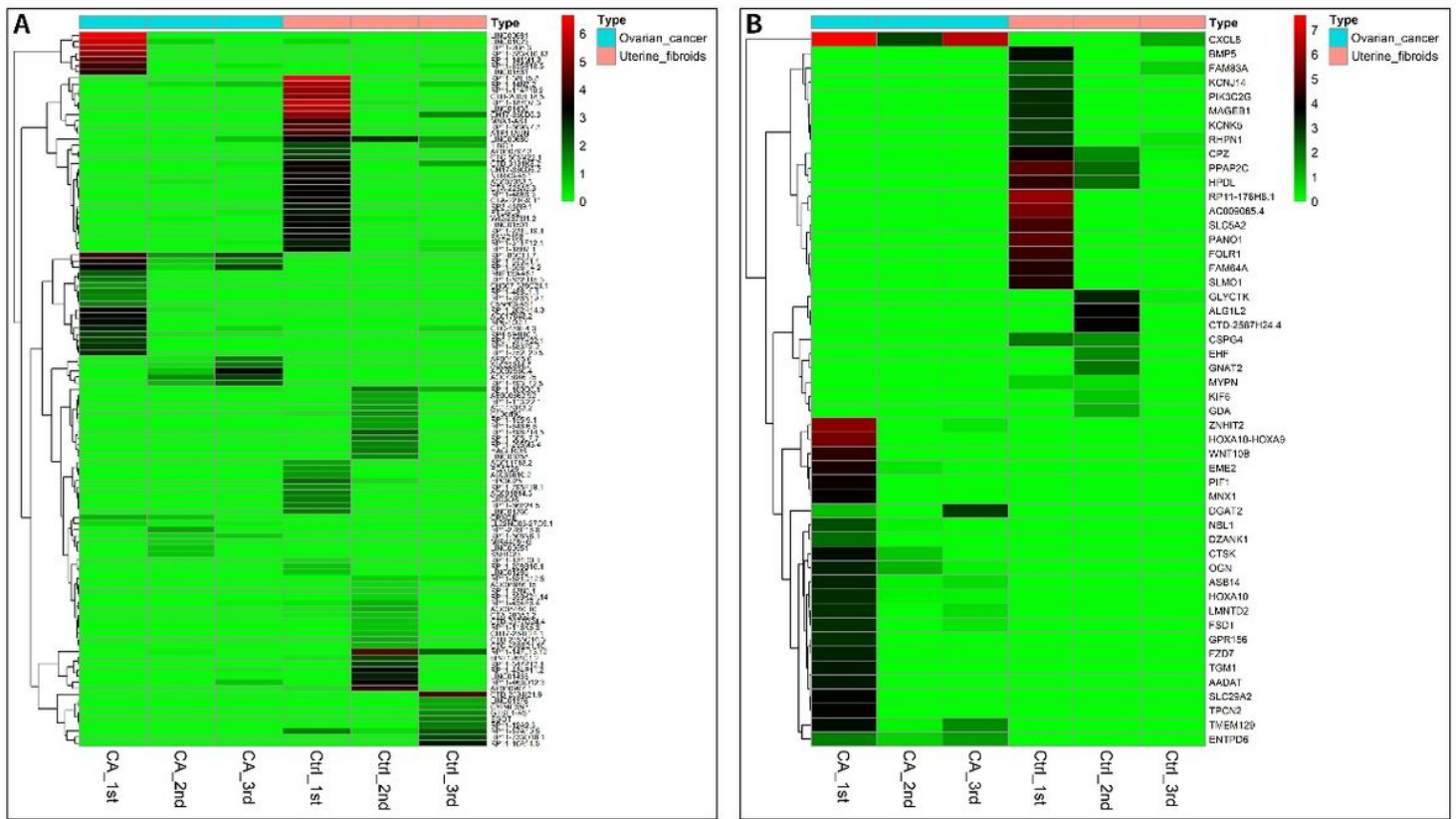# Figure 3

Heatmap of differentially expressed genes in ovarian cancer:A. Heatmap of 117 DElncRNAs clustering.B Top 50 most significant DEGs Note: tissue samples are presented as columns; individual genes are represented as rows. In patients with ovarian cancer, red indicates upregulated genes and green indicates downregulated genes. Top blue is listed as the ovarian cancer group and top pink is listed as the uterine fibroid group
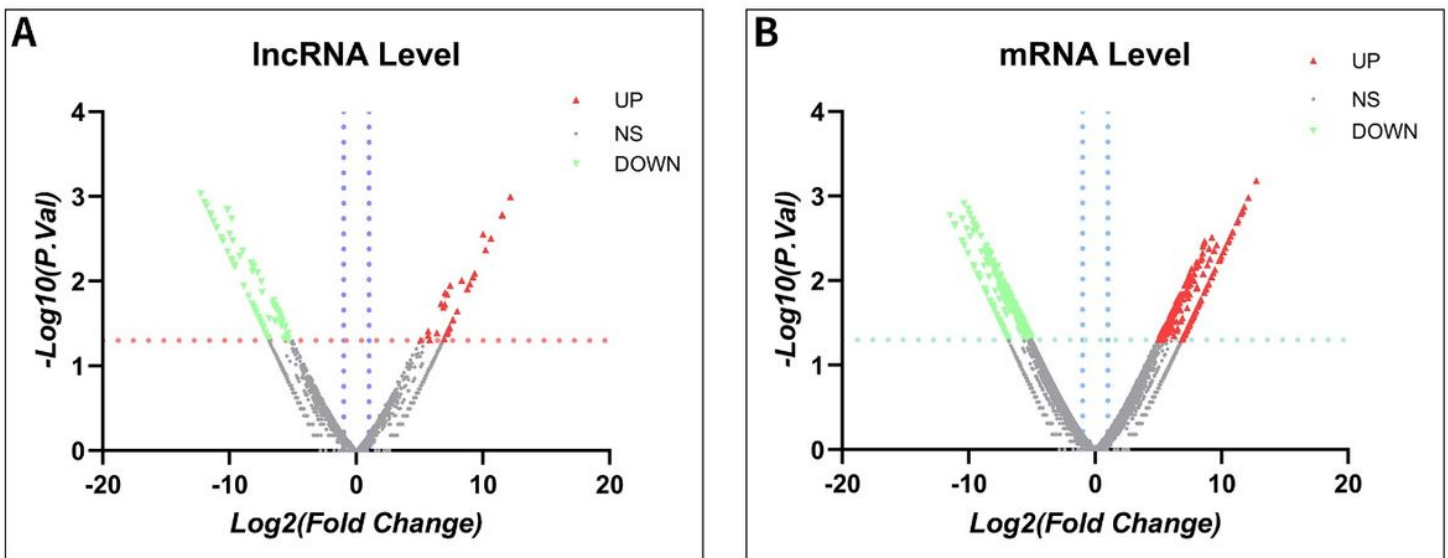
Figure 4

Volcano plots: A lncRNA volcano plots. B mRNA volcano plots Note: the vertical blue line corresponds to the up and down of log2FC, respectively, while the horizontal orange line indicates p-value < 0.05.red dots represent up-regulated and statistically significant DElncRNAs, green dots represent down regulated and statistically significant DElncRNAs. Fold change > 2.0 and P < 0.05 were used as standards, fold change was log2 transformed, and p-value was - log10 transformed
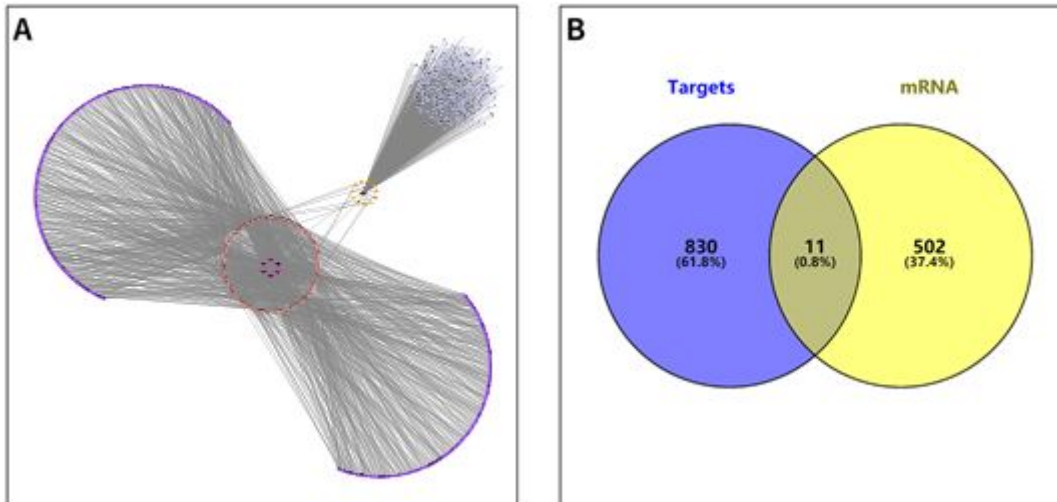

Figure 5

A the CeRNA interaction network of lncRNAs - miRNA – mRNA. B Venn diagram of predicted target genes and disease targets
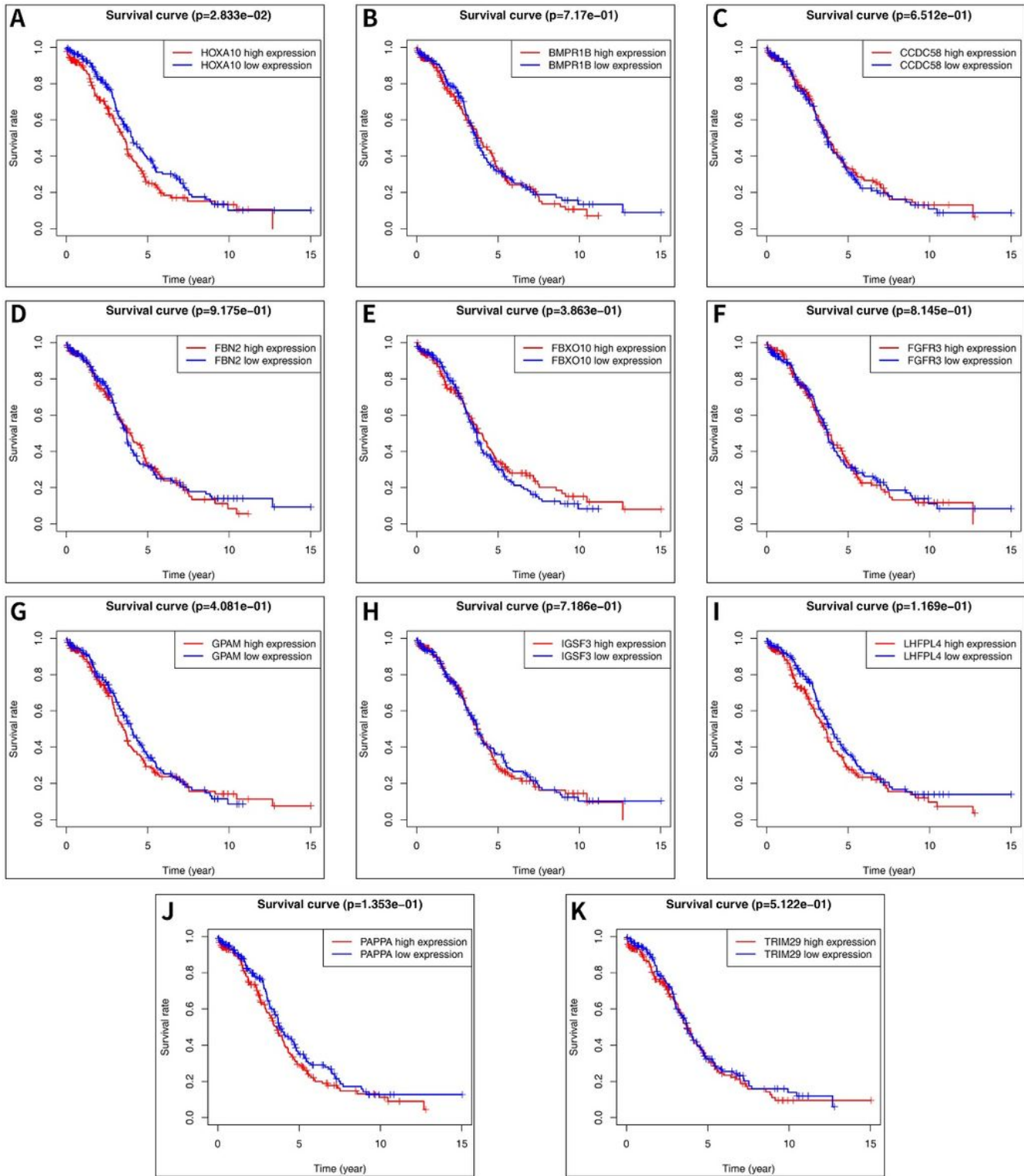
Figure 6

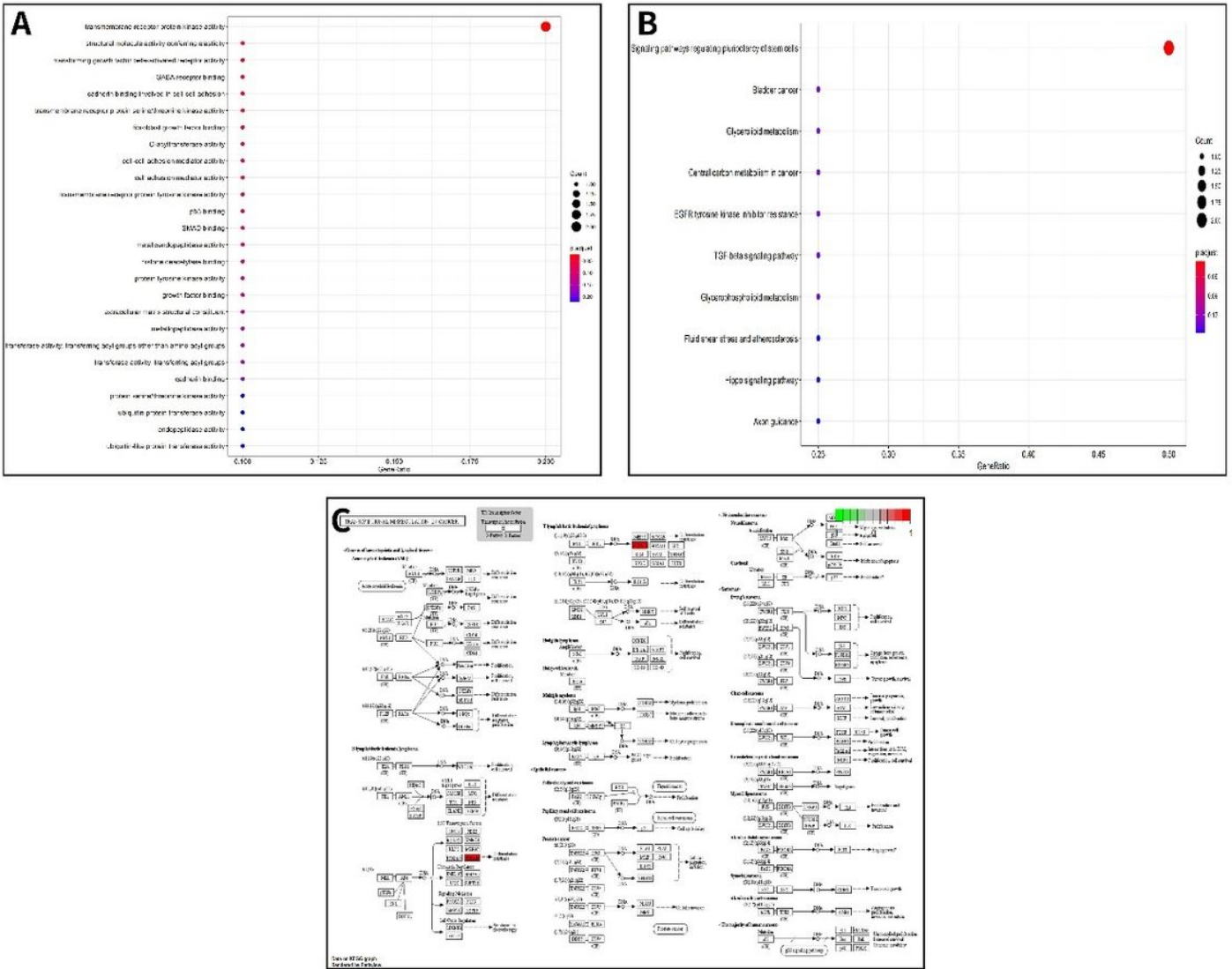Predicted target gene survival analysis curve

Figure 7

Go \ KEGG enrichment analysis:A potential target gene GO enrichment bubble plot. B potential target gene KEGG enrichment bubble plot.C Transcriptional misregulation in cancer signaling pathways