Original Article

ncRNA-mediated low expression of P2RY14 correlates with poor prognosis and tumor immune infiltration in ovarian carcinoma

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Background: Ovarian cancer (OV) has been puzzling clinicians because of its poor prognosis. More and more evidence show that the G protein coupled receptor P2RY14 plays a key role in the initiation and progression of various types of human cancer. The purpose of our study is to explore the correlation between P2RY14 and the prognosis of ovarian cancer patients and the relevant mechanism.

Methods: First, the differentially expressed gene P2RY14 was screened from The Cancer Genome Atlas (TCGA) database. Explored possible P2RY14 related miRNAs and lncRNAs through multiple public databases, predicted and analyzed the expression level of candidate miRNAs and candidate lncRNAs that can bind to candidate miRNAs in OV through StarBase database. The TIMER database was used to comprehensively analyze the expression of tumor infiltrating immune cells, and to analyze the correlation between the expression level of P2RY14 and the level of immune cell infiltration in OV or the expression level of immune checkpoints.

Results: Patients with P2RY14 overexpression had better overall survival (OS) and progression-free interval (PFI). In the Targetscan database, 22 upstream miRNAs that may bind to P2RY14 were predicted. According to the regulatory network constructed by the Cytoscape software, correlation analysis and the role of miRNAs in the prognosis of OV, we first determined that the candidate miRNAs were miR-34c-5p. Then, we predicted the upstream lncRNAs of miR-34c-5p in the StarBase database, the expression level of these lncRNAs in OV in the Gene Expression Profiling Interactive Analysis (GEPIA) database, and the role in prognosis. We determined that LINC00665 is the most potential lncRNA upstream of ovarian cancer miRNA (hsa-miR-34c-5p)-P2RY14. Then, we analyzed the results in the Timer database, suggesting that P2RY14 expression was positively correlated with $CD8^+$T Cell, $CD4^+$T Cell, Macrophage, Neutral and Dendritic cells, and negatively correlated with B cells. Meanwhile, P2RY14 was positively correlated with CD274 and PDCD1.

Conclusions: P2RY14 can be used as a new predictive biomarker of ovarian cancer. Intervention of P2RY14 can affect the prognosis of ovarian cancer by affecting LINC00665-miR-34c-5p-P2RY14 axis. These findings provide a potential target for the development of anti-cancer strategies for ovarian cancer.

Keywords: P2RY14; LINC00665; miR-34c-5p; ovarian cancer (OV); prognosis

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Introduction

Ovarian cancer (OV) is a highly aggressive tumor and the most prevalent female reproductive system malignancy (1). Annually, there are about 220,000 new cases of OV and over 140,000 fatalities worldwide (2). For tumors that are confined to the ovary, the effective treatment rate can reach 90%. However, the 5-year survival rate for OV is about 30%, and only 25% of patients may receive a diagnosis before their condition deteriorates (3). Even after surgery and chemotherapy, the overall survival (OS) rates of patients do not improve considerably; they are more likely to experience recurrence after surgery and acquire treatment resistance, which contributes to the high death rate of OV (4). The lack of identifiable tumor markers has become a serious issue in the early detection and monitoring of OV therapy.

The first purinoceptors, referred to known as P1 (adenosine) and P2 (ATP) receptors, were identified in 1978 (5). P2 (ATP) receptor is divided into P2X and P2Y, namely P2X (ion channel receptor) and P2Y (G protein coupled receptor). A subclass of G protein-coupled nucleotide receptors, known as P2Y receptors, primarily functions through adenine nucleoside triphosphate (ATP) (6). Thus far, nine functional P2Y receptors have been successfully cloned and confirmed using human tissue cells: P2Y1, 2, 4, 6, 11, 12, 13, and 14. The vacant numbers represent the P2Y receptor subtypes only found in non-mammals, whose function is unclear. The P2Y14 receptor is the only member of the P2 receptor family that reacts completely to uridine diphosphate glucose (UDP)-sugars, such as UDP-galactose and UDP-glucose (UDPG) (7). The up-regulation of the GPCR P2RY14 is associated with the activation of extracellular regulated protein kinases (ERK) signaling. It plays a role in patient survival, reducing interleukin-6 production in microgliomas and inhibiting glioma cell proliferation (8). The activity of P2RY14 via its ligand UDPG results in the selective phosphorylation of ERK1/2 (9). One study showed that P2RY14 could significantly prolong the OS of LUAD patients, which could be an important indicator of the prognosis of LUAD patients. New LUAD predictive biomarkers. P2RY14 may be related to LUAD immune invasion and play an important role in inhibiting tumor cell immune escape within LUAD microenvironment (10). However, at present, comprehensive research on the expression, prognosis, and mechanisms of P2RY14 in OV is insufficient. Moreover, the function of P2RY14 in tumor immune infiltration in OV is currently unknown.

With the rapid development of high-throughput sequencing technology, about 2% of ribonucleic acid (RNA) in mammalian genomes can encode proteins, and RNA that does not encode proteins is called non-coding RNA (ncRNA). ncRNA can be divided into two main categories according to length: <200 nucleotide ncRNAs, such as microRNA (miRNA) and small nuclear RNA (snRNA); >200 nucleotide ncRNAs, such as long non-coding RNA (lncRNA) and circular RNA (circRNA). Ncrnas are not only involved in the normal biological functions of the body, but also play an important role in the pathophysiological regulation of cells at various levels, such as epigenetics, transcription and post-transcription, which are further involved in the progression of a variety of diseases, especially in the occurrence and development of malignant tumors (11).

In the present study, we examined the relationship between P2RY14 expression and survival in various human cancer types. Subsequently, we investigated the

Highlight box

Key findings

- P2RY14 is down regulated in many types of human cancers (including OV), and OV patients with high P2RY14 expression have better prognosis. This paper studies and determines the upstream regulation mechanism of P2RY14 in OV, namely LINC00665-miR-34c-5p-P2RY14 axis.

What is known and what is new?

- Ovarian cancer is a highly invasive tumor. It is of great significance to reveal the potential molecular mechanism of OV carcinogenesis and progression.
- In OV, we also explored the ncRNA related regulation of P2RY14, including miRNA and lncRNA. This study suggests that P2RY14 expression is related to immune cell infiltration, immune cell biomarkers or OV immune checkpoint.

What is the implication, and what should change now?

- It needs to be further verified through basic experiments and clinical trials in the future.

regulation of P2RY14 by ncRNA, especially long non-coding RNA (lncRNA) and microRNA (miRNA), in OV. A correlation between P2RY14 expression, immune cell infiltration, biomarkers, and the OV immune checkpoint was established. This suggests that the ncRNA-mediated down-regulation of P2RY14 in OV patients is associated with a poor prognosis and immune involvement of the tumor. We present the following article in accordance with the REMARK reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6120/rc).

Methods

Downloading, processing, and analysis of The Cancer Genome Atlas (TCGA) data

From TCGA database (https://genome-cancer.ucsc.edu/), we acquired the messenger RNA (mRNA) expression data of different types of cancer. We subsequently standardized this data and used the R program (https://www.r-project.org/) to perform a differential expression analysis of P2RY14.

Gene Expression Profiling Interactive Analysis (GEPIA) database analysis

We used the GEPIA database (http://gepia.cancer-pku.cn/) to determine the expressions of P2RY14 and lncRNAs in various cancer types and assess the prognostic significance of potential lncRNAs in OV. This database was also used to evaluate the relationship between P2RY14 expression and immunological checkpoints in OV.

Candidate miRNA prediction

To understand whether upstream genes regulate P2RY14, we further explored related miRNAs that may be bound to the 3'UTR (three prime untranslated region) end of P2RY14 mRNA using the TargetScan database (https://www.TargetScan.org).

StarBase database analysis

Using the StarBase database (https://starbase.sysu.edu.cn), we conducted expression correlation analysis on the miRNA-P2RY14 and lncRNA-miR-34c-5p in OV. The miR-34c-5p expression levels in OV patients and healthy controls were also investigated. Additionally, potential lncRNAs that might bind to miR-34c-5p were predicted.

Tumor Immune Estimation Resource (TIMER) database analysis

Using the TIMER database (https://cistrome.shinyapps.io/timer/), we thoroughly examined the expression of immune cells that had infiltrated tumors and studied the association between immune checkpoint expression, immune cell infiltration, and P2RY14 expression levels in OV. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

As mentioned above, online resources were used to automatically calculate the statistical analysis for this investigation. A P value or log-rank P value of <0.05 was considered statistically significant.

Results

Expression of P2RY14 in different tumors

We evaluated the expression of P2RY14 in 33 human malignancies from TCGA database to investigate its potential role in carcinogenesis. P2RY14 was considerably up-regulated compared to standard samples in liver hepatocellular carcinoma (LIHC) and kidney renal clear cell carcinoma (KIRC). At the same time, it was significantly down-regulated in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), kidney chromophobe (KICH), kidney renal papillary cell carcinoma (KIRP), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC) (*Figure 1A*). Then, using the GEPIA database, the level of P2RY14 in various tumors was also investigated. We discovered that P2RY14 was up-regulated in LAML (acute myeloid leukemia) but down-regulated in LUSC, LUAD, BLCA, COAD, and READ (*Figure 1B*).

The prognosis of P2RY14 in different tumors

Using the TCGA database, we also explored the relationship between P2RY14 and several tumor prognoses, including OS

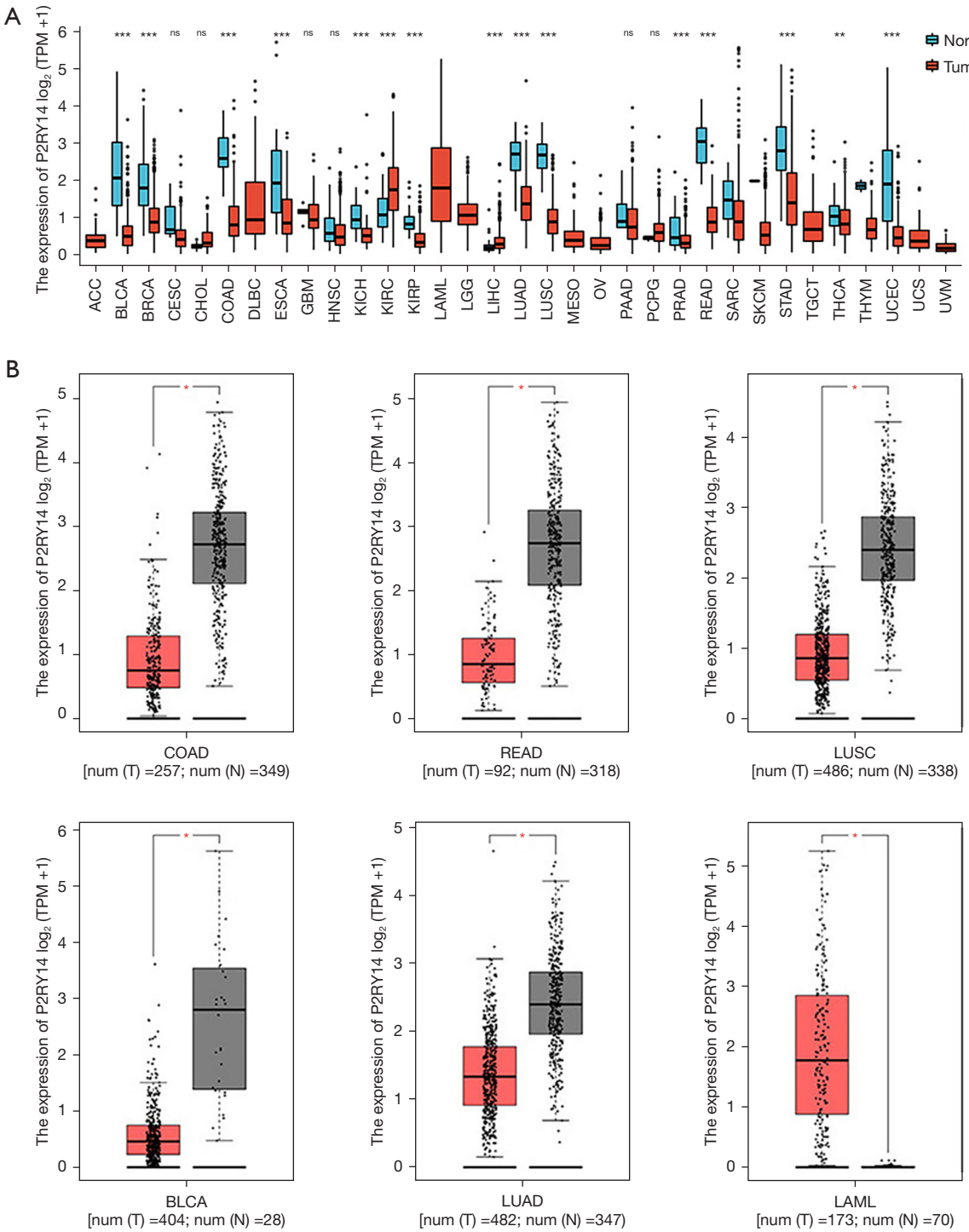

Figure 1 Expression of P2RY14 in several tumor types. P2RY14 expression in various malignancies found in TCGA database (A); P2RY14 expression in various tumors found in the GEPIA database (B). *, P<0.05; **, P<0.01; ***, P<0.001; ns, not significant. TPM, transcripts per million; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma; LUSC, lung squamous cell carcinoma; BLCA, bladder urothelial carcinoma; LUAD, lung adenocarcinoma; LAML, acute myeloid leukemia; TCGA, The Cancer Genome Atlas; GEPIA, Gene Expression Profiling Interactive Analysis.

and progression-free survival [or progression-free interval (PFI)]. Individuals with high P2RY14 expression in the LUAD, KIRC, OV, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), sarcoma (SARC), and oral squamous cell carcinoma (OSCC) had a superior OS. However, those with elevated expression in the BLCA and STAD had a worse OS, as shown in *Figure 2A*. Next, we observed that patients with low expression of P2RY14 in testicular germ cell tumors (TGCT), STAD, and ESCC had a better PFI, while those with high P2RY14 expression in CESC, KIRC, and OV had a better PFI (*Figure 2B*).

P2RY14 upstream miRNA identification and validation

It is widely believed that ncRNAs control how genes are expressed. We initially anticipated the upstream miRNAs that would bind to P2RY14 in the TargetScan database, and ultimately discovered 22 candidate miRNAs to determine whether certain ncRNAs regulate P2RY14. The details of these miRNAs are shown in *Table 1*. Cytoscape software was used to create a miRNA-P2RY14 regulatory network to improve visualization (*Figure 3*). There must be a negative relationship between miRNA and P2RY14 based on how miRNA regulates the expression of its gene products. As a result, we analyzed expression correlation in the StarBase database, as presented in *Table 2*. At the same time, we constructed a scatter plot to express this correlation more intuitively (*Figure 4*). We initially screened and obtained 13 negatively correlated candidate miRNAs. The expression of putative miRNAs with prognostic significance in the prediction of OV was examined using the StarBase database (*Figure 5*). We identified several potential miRNAs, including hsa-miR-4770 and hsa-miR-34c-5p. Finally, we chose hsa-miR-34c-5p as our final candidate miRNA based on previously published research results.

LncRNA prediction and analysis upstream of miR-34c-5p

Next, Cytoscape software was used to generate a lncRNA-miR-34c-5p regulatory network using the TargetScan database to determine the upstream lncRNA of miR-34c-5p (*Figure 6*). Based on the assumption that the expression differential of lncRNAs in OV and surrounding tissues was statistically significant according to the binding of base pairs, we preliminarily retrieved six potential lncRNAs at the same time. The specifics of these lncRNAs are displayed in *Table 3*. Using the GEPIA database, we then calculated the overexpression of these lncRNAs in OV. LINC00665, LINC01123, and LINC01132 were considerably up-regulated in OV tumor tissues compared to the normal controls, but LINC00667, LINC00921, and NEAT1 were significantly down-regulated (*Figure 7*). The predictive significance of these six lncRNAs in OV was then assessed in the StarBase database. Only LINC00665 and LINC01132 were quite different, and the prognosis for OV patients with elevated levels of both genes was favorable (*Figure 8*). In the competitive endogenous RNA (ceRNA) concept, lncRNAs can efficiently enhance mRNA expression by adhering to conserved miRNAs. Therefore, there must be a negative association between miRNA and lncRNA, as demonstrated in *Table 4* and *Figure 9*.

Furthermore, the correlation between these two lncRNAs and miR-34c-5p in OV was determined using the StarBase database. The results of the expression, survival, and correlation analyses showed that LINC00665 is a promising lncRNA upstream of the OV miRNA (hsa-miR-34c-5p)-P2RY14.

Correlation analysis between P2RY14 in the TIMER database and immune cell infiltration in OV

A previous study revealed that P2RY14 also has a role in the immune system. The activity and mechanisms of P2RY14 can be better understood through correlation analysis. Therefore, we examined P2RY14 in OV and immune cell invasion in the TIMER database. P2RY14 expression was positively correlated with dendritic cells, neutrophils, macrophages, $CD8^+$ T-cells, and $CD4^+$ T-cells but negatively associated with B-cells (*Figure 10*).

Relationship between P2RY14 and immune cell biomarker expression in OV

To broadly comprehend the significance of P2RY14 in tumor immunity, we compared P2RY14 expression with immune cell markers in OV using the GEPIA database. As demonstrated in *Table 5*, dendritic cells (NRP1, CD1C, HLA-DPA1, HLA-DQB1, HLA-DPB1, and HLA-DRA), M1 biomarkers (IRF5, PTGS2, and NOS2), M2 biomarkers (VSIG4), B-cell biomarkers (CD79A), and M2 biomarkers (ITGAM) were all positively associated.

Connection between immunological checkpoints and P2RY14 in OV

Important immunological checkpoints are responsible for

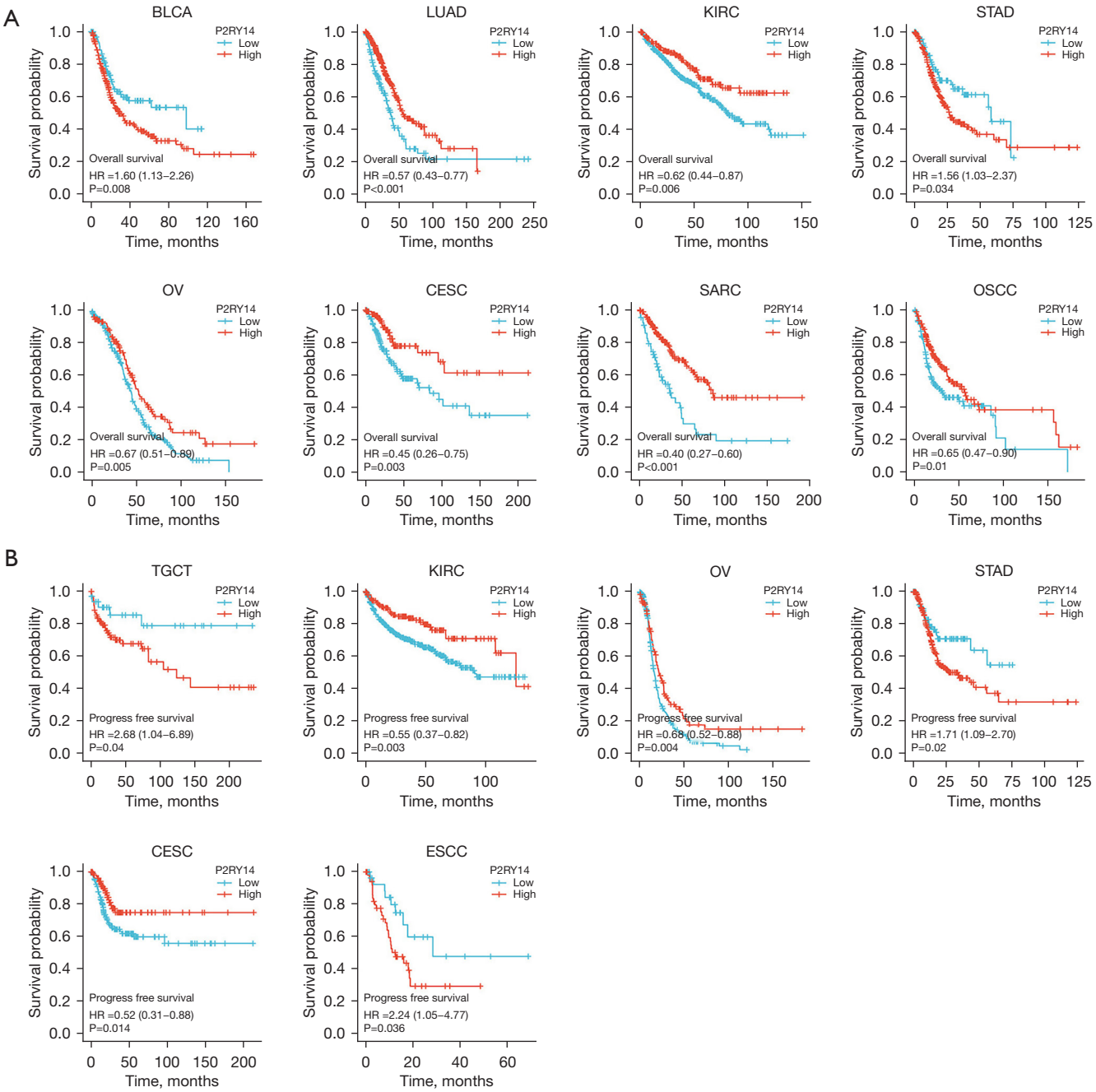

Figure 2 P2RY14 prognosis in several tumor types from TCGA database. (A) The association between P2RY14 expression and OS in various malignancies; (B) the association between PFI and P2RY14 expression in various cancers. P<0.05 was considered statistically significant. BLCA, bladder urothelial carcinoma; LUAD, lung adenocarcinoma; KIRC, kidney renal clear cell carcinoma; STAD, stomach adenocarcinoma; OV, ovarian cancer; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; SARC, sarcoma; OSCC, oral squamous cell carcinoma; TGCT, testicular germ cell tumors; ESCC, European Space Components Coordination; TCGA, The Cancer Genome Atlas; OS, overall survival; PFI, progression-free interval.

Table 1 Predicted miRNAs in the TargetScan database that bind to the 3'UTR end of P2RY14

Candidate miRNA	Position in the UTR	Seed match	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT	Predicted relative KD
hsa-miR-140-5p	285–292	8mer	−0.46	99	−0.46	0.233	<0.1	−3.823
hsa-miR-449a	106–113	8mer	−0.48	98	−0.48	0.131	<0.1	−4.531
hsa-miR-449b-5p	106–113	8mer	−0.49	98	−0.49	0.131	<0.1	−4.221
hsa-miR-34a-5p	106–113	8mer	−0.5	98	−0.5	0.131	<0.1	−4.639
hsa-miR-34c-5p	106–113	8mer	−0.52	98	−0.52	0.131	<0.1	−4.221
hsa-miR-129-2-3p	453–460	8mer	−0.39	98	−0.39	1.654	<0.1	−4.019
hsa-miR-129-1-3p	453–460	8mer	−0.39	98	−0.39	1.654	<0.1	−4.019
hsa-miR-143-3p	96–102	7mer-m8	−0.32	98	−0.32	1.554	0.23	−4.053
hsa-miR-4770	96–102	7mer-m8	−0.32	98	−0.32	1.554	0.23	−4.076
hsa-miR-6088	96–102	7mer-m8	−0.27	97	−0.27	1.554	0.23	−4.104
hsa-miR-219a-5p	529–536	8mer	−0.34	96	−0.34	0.048	<0.1	−4.895
hsa-miR-6766-3p	529–536	8mer	−0.34	96	−0.34	0.048	<0.1	−5.152
hsa-miR-4782-3p	529–536	8mer	−0.34	96	−0.34	0.048	<0.1	−5.152
hsa-miR-106b-5p	630–636	7mer-m8	−0.16	92	−0.16	0.403	<0.1	−4.427
hsa-miR-20b-5p	630–636	7mer-m8	−0.13	91	−0.13	0.403	<0.1	−5.412
hsa-miR-106a-5p	630–636	7mer-m8	−0.15	91	−0.15	0.403	<0.1	−5.029
hsa-miR-20a-5p	630–636	7mer-m8	−0.15	91	−0.15	0.403	<0.1	−4.491
hsa-miR-17-5p	630–636	7mer-m8	−0.14	91	−0.14	0.403	<0.1	−5.537
hsa-miR-9-5p	15–21	7mer-1A	−0.16	91	−0.16	0.066	<0.1	−2.103
hsa-miR-302e	629–635	7mer-m8	−0.16	90	−0.16	0.403	<0.1	−4.39
hsa-miR-372-3p	629–635	7mer-m8	−0.18	90	−0.18	0.403	<0.1	−4.965
hsa-miR-93-5p	630–636	7mer-m8	−0.12	90	−0.12	0.403	<0.1	−5.486

UTR, untranslated region; PCT, percentage; KD, k-dimensional.

tumor immune escape, including cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed death receptor 1/programmed death receptor ligand-1 (PD1/PD-L1). So, we assessed the connection between P2RY14 and PD-L1, CTLA-4, or PD1. We discovered that CD274 and programmed cell death 1 (PDCD1) had strong positive correlations with P2RY14. We also identified a substantial positive association between P2RY14, CD274, and PDCD1 in OV, which was similar to the TIMER database analysis results (*Figure 11*). These findings imply that P2RY14-mediated OV carcinogenesis may involve tumor immune escape.

Discussion

At present, OV is still associated with a poor prognosis. Understanding the molecular process of P2RY14 could offer important insights for designing effective treatment targets or identifying potential prognostic indicators. Increasing evidence suggests that P2RY14 may be essential for the onset and development of numerous cancers and illnesses. However, research on P2RY14 in OV is still lacking; thus, further investigation and exploration are required. In this study, TCGA database was utilized to analyze P2RY14 expression in a wide variety of cancers. Then, the GEPIA

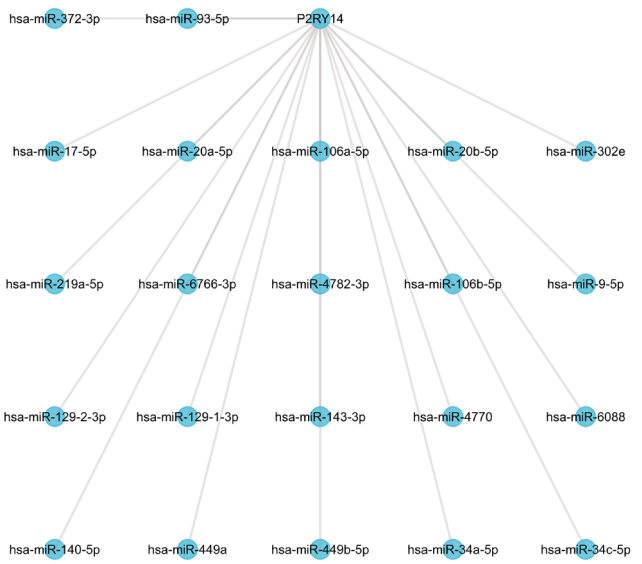

Figure 3 Cytoscape-generated miRNA-P2RY14 regulatory network.

database was employed to validate our findings. At the same time, we discovered that the P2RY14 expression was up-regulated in paracancerous tissues, and individuals with high P2RY14 expression in OV had an improved OS and PFI. These findings reflect the significance of our study on target molecules.

Circular RNAs (circRNAs), miRNAs, lncRNAs, and other ncRNAs interact via the ceRNA mechanism and control gene expression, as has been well-established (12-16). After estimating the upstream miRNAs that could attach to P2RY14 in the TargetScan database, we discovered 22 potential miRNAs. These candidates had various expressions and functions in OV, including overexpression of the lncRNA H19. We found that the miR-140-5p gene was down-regulated and the phosphatidylinositide 3-kinases/protein kinase B (PI3K/AKT) signaling pathway was activated, encouraging OV cells to invade, migrate, and undergo epithelial-mesenchymal transition (17). MicroRNA-449a down-regulates NOTCH1 in OV cells, which decreases cell survival and improves the effects of cisplatin-induced cytotoxicity by platinum (18). Through the control of mitogen-activated protein kinase 3 (MAP3K3) by miR-129-3p, circSETDB1 knockdown prevents the malignant evolution of serous OV (19). Also, the transforming growth factor-β activating kinase 1 (TAK1) axis, which is activated by the lncRNA MCM3AP antisense RNA 1 (non-protein coding) (MCM3AP-AS1), induces OV progression (20).

Table 2 Correlation between the candidate miRNAs in the StarBase database and P2RY14 in ovarian cancer

Candidate miRNA	Gene	R value	P value
hsa-miR-34a-5p	P2RY14	0.001	9.86e-1
hsa-miR-449b-5p	P2RY14	−0.225	1.10e-5
hsa-miR-449a	P2RY14	−0.199	1.00e-4
hsa-miR-140-5p	P2RY14	0.073	1.58e-1
hsa-miR-34c-5p	P2RY14	−0.046	3.72e-1
hsa-miR-129-2-3p	P2RY14	0.028	5.84e-1
hsa-miR-129-1-3p	P2RY14	−0.066	2.02e-1
hsa-miR-143-3p	P2RY14	0.131	1.09e-2
hsa-miR-4770	P2RY14	−0.096	6.22e-2
hsa-miR-6088	P2RY14	0.000	1.00e+0
hsa-miR-4782-3p	P2RY14	0.073	1.60e-1
hsa-miR-6766-3p	P2RY14	0.058	2.60e-1
hsa-miR-219a-5p	P2RY14	−0.106	3.95e-2
hsa-miR-106b-5p	P2RY14	−0.163	1.52e-3
hsa-miR-9-5p	P2RY14	−0.034	5.15e-1
hsa-miR-17-5p	P2RY14	−0.102	4.73e-2
hsa-miR-20a-5p	P2RY14	−0.108	3.59e-2
hsa-miR-372-3p	P2RY14	−0.094	6.94e-2
hsa-miR-302e	P2RY14	0.000	1.00e+0
hsa-miR-20b-5p	P2RY14	−0.071	1.71e-1
hsa-miR-106a-5p	P2RY14	0.002	9.63e-1
hsa-miR-93-5p	P2RY14	−0.022	6.75e-1

After correlation and prognostic analysis, we found that hsa-miR-34c-5p and hsa-miR-4770 were our potential candidate miRNAs. Referring to relevant research reports, we found that there are few studies on miR-4770 at present, and tumor related studies are only colorectal cancer. However, there are abundant researches on miR-34c-5p, for example, the upregulation of miR-34c-5p inhibits nasopharyngeal carcinoma cells through mediating NOTCH1 (21). MiR-34c-5p mediates the malignant behavior of oral squamous cell carcinoma through targeting and TRIM29 (22). LncRNA-ES3/miR-34c-5p/BMF axis participates in regulating VSMC calcification/senescence induced by high glucose (23). At the same time, a study reported that the characteristics of ovarian cancer extracellular vesicle miRNA (OCEM) developed based on miRNAs including miR-34c-5p can improve the

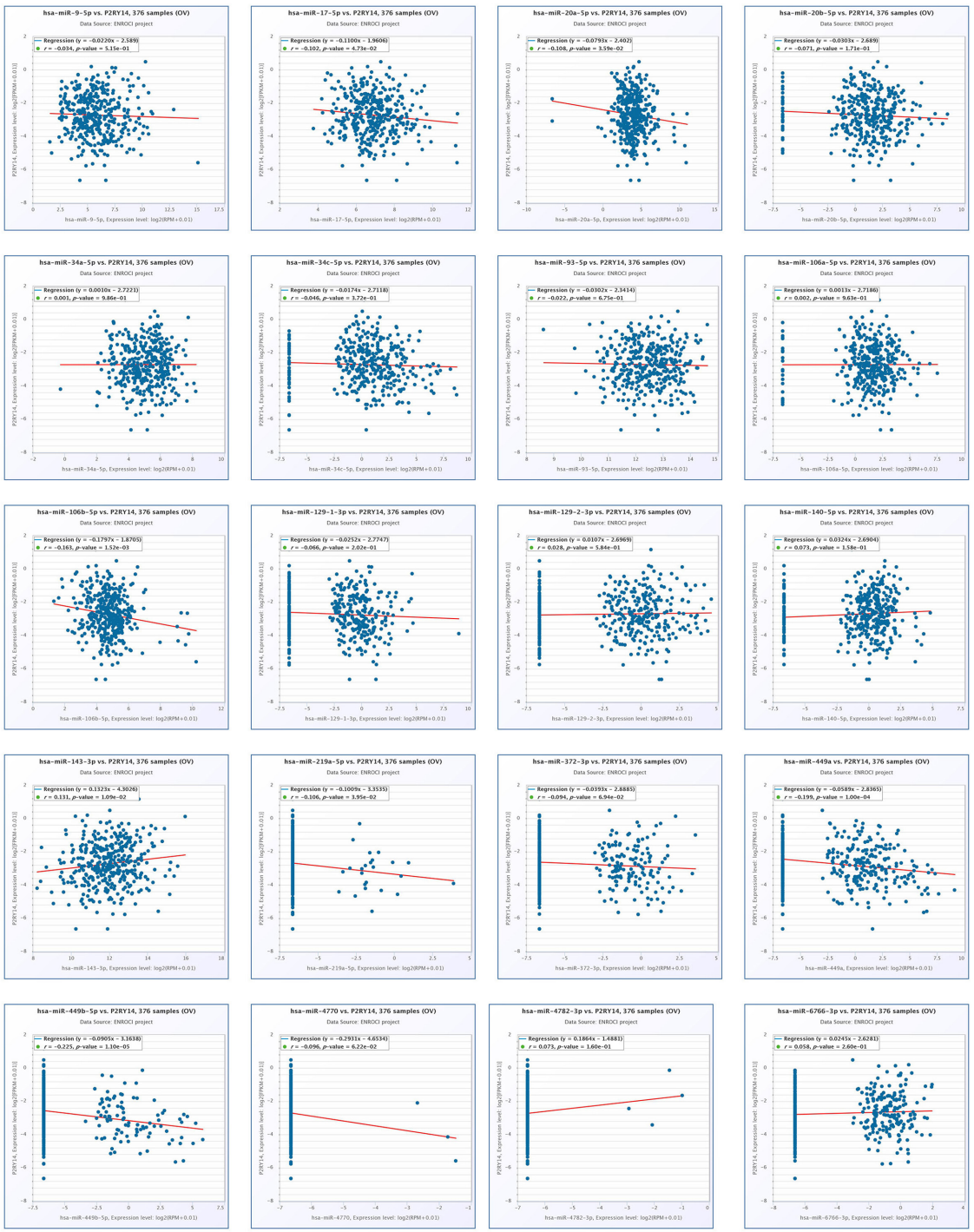

Figure 4 Correlation between miRNA and P2RY14 in the StarBase database. P<0.05 was considered statistically significant. OV, ovarian cancer; ENROCI, The Encyclopedia of RNA Interactomes (http://starbase.sysu.edu.cn/index.php); FPKM, fragments per kilobase of exon model per million mapped fragments; RPM, reads per million mapped reads.

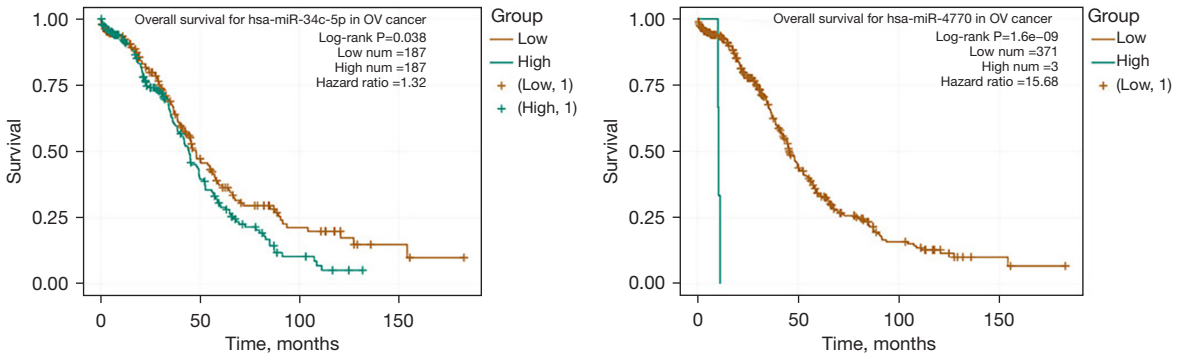

Figure 5 Expression of candidate miRNAs in the StarBase database in the prognosis of OV. P<0.05 was considered statistically significant. OV, ovarian cancer.

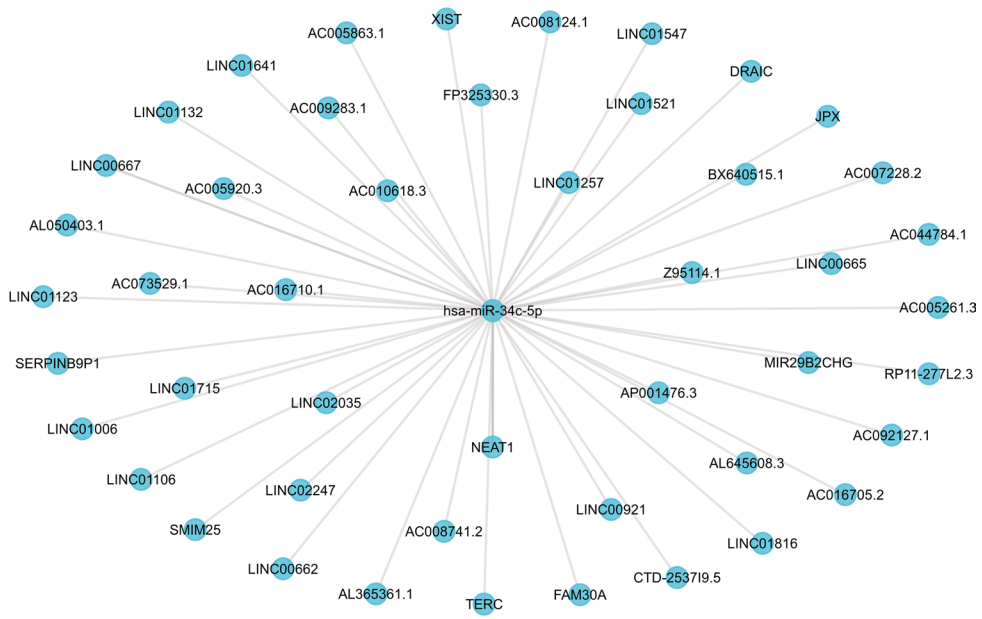

Figure 6 Cytoscape software was used to design the lncRNA-miR-34c-5p regulatory network.

Table 3 Upstream lncRNAs predicted to regulate hsa-miR-34c-5p in ovarian cancer from the TargetScan database

Gene ID	Gene name	clipExpNum	RBP	merClass	miRseq	Align	pancancerNum
ENSG00000232677	LINC00665	5	AGO1-4	7mer-m8	cguUAGUC--GAUUGAU-GUGACGGa	| ||| |: ||: ||||||	6
ENSG00000204588	LINC01123	4	AGO1-4, AGO2	7mer-m8	cguUAGUCGAUUGAUGUGACGGa	||||| |:| ||||||	5
ENSG00000227630	LINC01132	1	AGO1-4	8mer	cgUUAGUCGAUUGAUGUGACGGa	|| ::| ::|| ||||||	3
ENSG00000245532	NEAT1	4	AGO1-4, AGO2	7mer-m8	cguuaGUCGAUUGAUGUGACGGa	|||| ||:||||||	3
ENSG00000263753	LINC00667	3	AGO2	7mer-m8	cgUUAGUCGAU-UG---AUGUGACGGa	|||| ||| :| |:||||||	2
ENSG00000281005	LINC00921	1	AGO2	7mer-m8	cguUAGUC-GAUU---GAUGUGACGGa	||||| |||| | :||||||	1

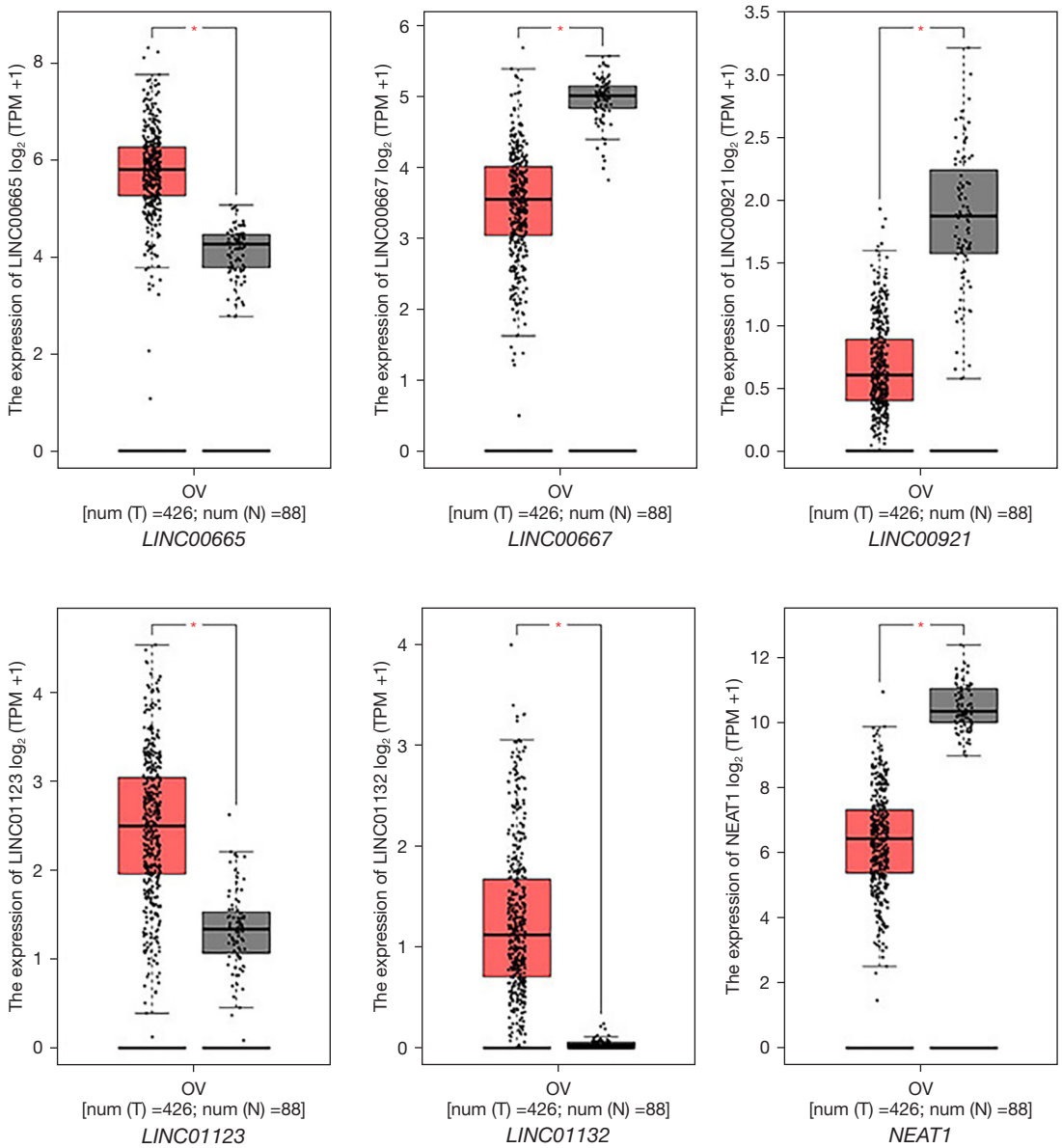

Figure 7 Expression of candidate lncRNAs in OV from the GEPIA database. *, P<0.05. TPM, transcripts per million; OV, ovarian cancer; GEPIA, Gene Expression Profiling Interactive Analysis.

diagnosis and treatment of ovarian cancer (24). At the same time, the malignant ascites derived extracellular vesicles can significantly promote the invasion of ovarian cancer cells and the growth of ascites derived organs (25). After analyzing the correlation and predictive data, we ultimately decided that miR-34c-5p was the best potential miRNA. Next, we projected the upstream lncRNAs and identified LINC00665 as the most likely lncRNA upstream of OV miRNA (hsa-miR-34c-5p)-P2RY14 through correlation, survival, and expression analyses. Additionally, substantial evidence suggests that the lncRNA LINC00665 targets miR-181a-5p/FH2 domain containing (FHDC) and stimulates OV cell proliferation while inhibiting apoptosis (26). Furthermore, a possible OV regulatory mechanism was found to involve the LINC00665-miR-34c-5p-P2RY14 axis.

Numerous studies have shown that tumor immune cell invasion affects the effectiveness of chemotherapy, immunotherapy, or radiation as well as the survival of

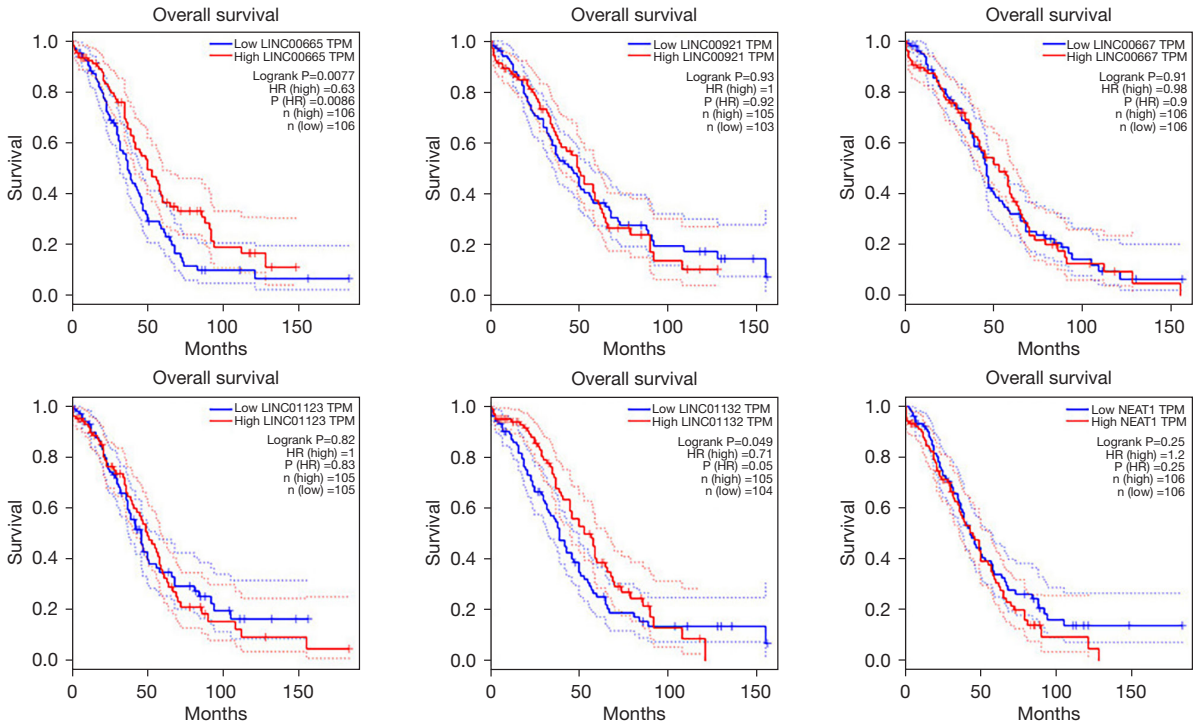

Figure 8 Correlation between candidate lncRNAs in the StarBase database and the prognosis of OV patients. P<0.05 was considered statistically significant. TPM, transcripts per million; HR, hazard ratio; OV, ovarian cancer.

Table 4 Results of a correlation study of predicted lncRNA and miRNA (hsa-miR-34c-5p)

lncRNA	miRNA	R value	P value
LINC00665	hsa-miR-34c-5p	–0.013	7.96e-1
LINC01132	hsa-miR-34c-5p	0.093	7.25e-2

P<0.05 was considered statistically significant.

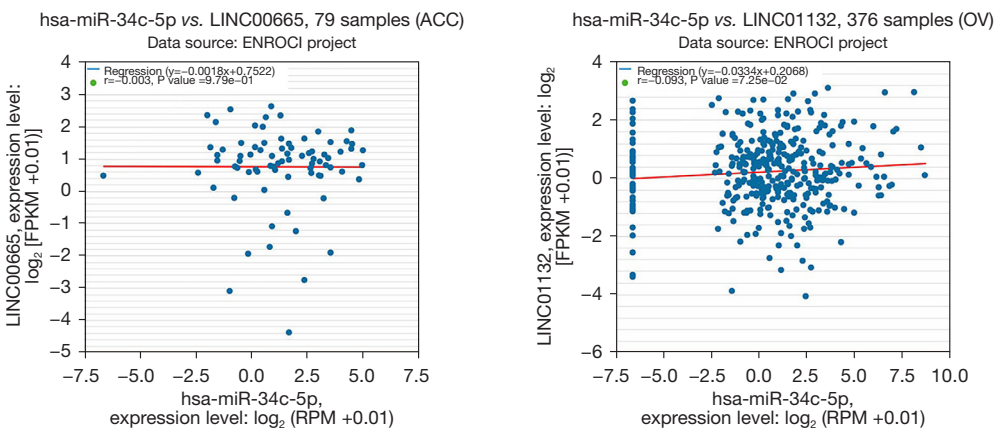

Figure 9 Correlation between LINC00665 and LINC01132 and miR-34c-5p in the StarBase database. P<0.05 was considered statistically significant. ACC, adrenocortical carcinoma; OV, ovarian cancer; ENROCI, The Encyclopedia of RNA Interactomes (http://starbase.sysu.edu.cn/index.php); FPKM, fragments per kilobase of exon model per million mapped fragments; RPM, reads per million mapped reads.

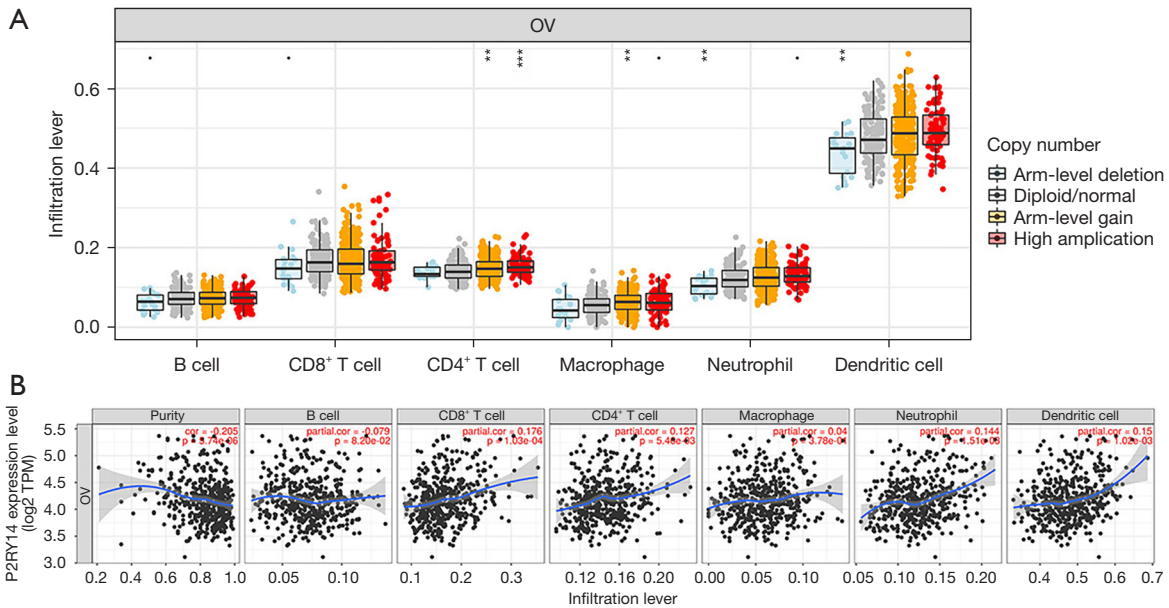

Figure 10 Analysis of the correlation between P2RY14 and immune cell invasion in OV from the TIMER database. **, P<0.01; ***, P<0.001 were considered statistically significant. OV, ovarian cancer; TPM, transcripts per million.

Table 5 GEPIA database investigation of the relationship between immune cell markers and P2RY14 in ovarian cancer

Immune cell	Biomarker	R value	P value
B cell	CD19	0.0033	0.95
	CD79A	0.21	7.8e-06
CD8+ T cell	CD8A	0.45	0
	CD8B	0.043	0.38
CD4+ T cell	CD4	0.43	0
M1	IRF5	0.13	0.007
	PTGS2	0.15	0.0023
	NOS2	0.12	0.012
M2	CD163	0.44	0
	VSIG4	0.36	7.5e-15
	MS4A4A	0.61	0
Neutrophils	CEACAM8	0.06	0.22
	ITGAM	0.38	4.4e-16
	CCR7	0.47	0
Dendritic cell	CD1C	0.32	1.9e-11
	HLA-DPA1	0.27	8.8e-09
	HLA-DRA	0.26	6.3e-08
	HLA-DQB1	0.18	0.00022
	HLA-DPB1	0.29	1.4e-09
	NRP1	0.29	1.5e-09
	ITGAX	0.39	0

GEPIA, Gene Expression Profiling Interactive Analysis.

cancer patients (27-29). Our findings indicate a strong association between P2RY14 and various immune system cells in OV, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. Additionally, P2RY14 was strongly linked to these invading immune cell indicators. These results imply that the function of P2RY14 in OV could be influenced by tumor immune participation.

Also, proper immunological checkpoint expression is necessary for immunotherapy effectiveness as well as the infiltration of immune cells into the tumor microenvironment (30). Consequently, the relationship between P2RY14 and immunological checkpoints was also investigated. We discovered that P2RY14 was significantly linked to CD274 and PDCD1, which further substantiated the idea that focusing on P2RY14 could improve the effectiveness of immunotherapy in OV.

Conclusions

In summary, our research shows that P2RY14 is down-regulated in various human malignancies, including OV, and that OV patients with high P2RY14 expression levels tend to have better prognoses. We determined the LINC00665-miR-34c-5p-P2RY14 axis as the upstream regulation mechanism of P2RY14 in OV. However, according to our most recent research, P2RY14 may modify the tumor

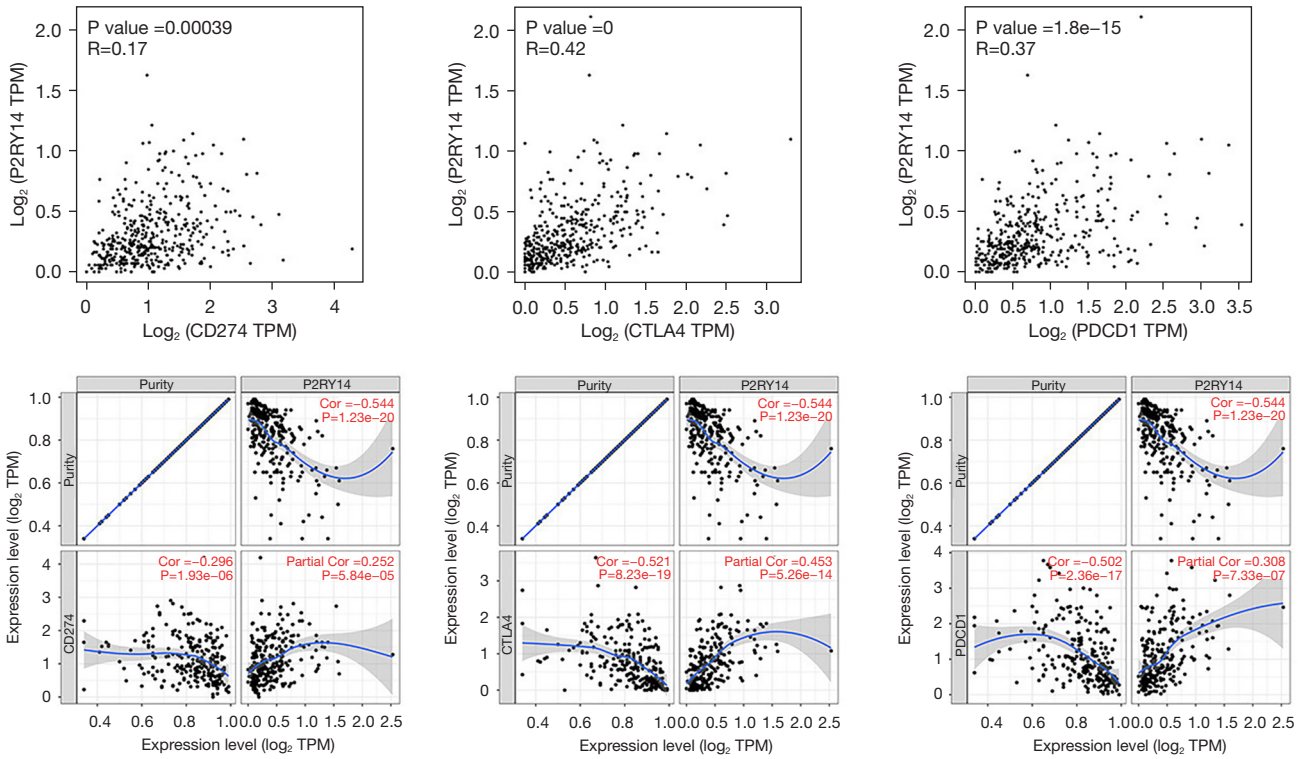

Figure 11 Relationship between P2RY14 expression and CD274, CTLA4, and PDCD1 expression in OV. P<0.05 was considered statistically significant. TPM, transcripts per million; OV, ovarian cancer.

immunological microenvironment, which may impact the prognosis of OV. Therefore, in the future, basic research and clinical studies will be needed to confirm the current evidence.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6120/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6120/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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