A Comprehensive Analysis of the Downregulation of \textit{miRNA-1827} and Its Prognostic Significance by Targeting \textit{SPTBN2} and \textit{BCL2L1} in Ovarian Cancer

Penghui Feng\textsuperscript{1†}, Zhitong Ge\textsuperscript{2†}, Zaixin Guo\textsuperscript{1}, Lin Lin\textsuperscript{1,3} and Qi Yu\textsuperscript{1*}

\textsuperscript{1}Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, \textsuperscript{2}Department of Ultrasound, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, \textsuperscript{3}Department of Obstetrics and Gynecology, The Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Beijing, China

\textbf{Background:} Previous studies demonstrated that \textit{miRNA-1827} could repress various cancers on proliferation, angiogenesis, and metastasis. However, little attention has been paid to its role in ovarian cancer as a novel biomarker or intervention target, especially its clinical significance and underlying regulatory network.

\textbf{Methods:} A meta-analysis of six microarrays was adopted here to determine the expression trend of \textit{miRNA-1827}, and was further validated by gene expression profile data and cellular experiments. We explored the functional annotations through enrichment analysis for the differentially expressed genes targeted by \textit{miRNA-1827}. Subsequently, we identified two hub genes, \textit{SPTBN2} and \textit{BCL2L1}, based on interaction analysis using two online archive tools, miRWALK (it consolidates the resources of 12 miRNA-focused servers) and Gene Expression Profiling Interactive Analysis (GEPIA). Finally, we validated their characteristics and clinical significance in ovarian cancer.

\textbf{Results:} The comprehensive meta-analysis revealed that \textit{miRNA-1827} was markedly downregulated in clinical and cellular specimens. Transfection of the \textit{miRNA-1827} mimic could significantly inhibit cellular proliferation. Concerning its target genes, they were involved in diverse biological processes related to tumorigenesis, such as cell proliferation, migration, and the apoptosis signaling pathway. Moreover, interaction analysis proved that two hub genes, \textit{SPTBN2} and \textit{BCL2L1}, were highly associated with poor prognosis in ovarian cancer.

\textbf{Conclusion:} These integrated bioinformatic analyses indicated that \textit{miRNA-1827} was dramatically downregulated in ovarian cancer as a tumor suppressor. The upregulation of its downstream modulators, \textit{SPTBN2} and \textit{BCL2L1}, was associated with an unfavorable prognosis. Thus, the present study has identified \textit{miRNA-1827} as a potential intervention target for ovarian cancer based on our bioinformatic analysis processes.

\textbf{Keywords:} \textit{miRNA-1827}, microRNA-1827, SPTBN2, BCL2L1, Ovarian Cancer
INTRODUCTION

Ovarian cancer (OV), as one of the three major gynecological malignancies, poses a severe threat to women’s life quality and reproductive health (Siegel et al., 2019). Annually, there are ~300,000 new cases worldwide identified as OV, with the highest mortality rate among all gynecological malignancies (Bray et al., 2018). OV is classified into various histopathological types, and epithelial cancer is the most common type. Currently, nearly 65–75% of patients are already in the middle or advanced stages when they are first confirmed because the tumor lesions are always located deep in the pelvic cavity, and there are no practical approaches for screening and diagnosis for early onset. Although ~75% of patients can benefit from the initial treatment with impermanent remission, almost all terminal cases will develop into the recurrent and multidrug-resistant status, and the 5-year survival rate of OV patients is merely 30–50% (Salani et al., 2017; Allemanni et al., 2018).

Recently, the development of bioinformatics has promoted the exploration of various kinds of cancers by integrating data resources and clinical information, which assists experimental biologists in carrying forward these findings into clinical validation and application (Fu et al., 2020; Udhaya Kumar et al., 2020a). Previous studies have determined the correlation between the aberrant expression of specific genes and oncogenesis and progression of OV, and unveiled the predictive potential of these biomarkers for prognosis (Zheng et al., 2019; Yang et al., 2020). As reported, a study has analyzed energy metabolism–associated characteristics to evaluate the prognosis of patients with OV by nonnegative matrix factorization clustering analysis; it finally established an eight-gene signature associated with metabolic genes (Wang and Li, 2020). Another study has also identified four core genes associated with the prognosis of OV using the Analyze Networks algorithm (Kumar et al., 2019). At the same time, mounting evidence has revealed the latent significance of miRNAs as new diagnostic and prognostic markers based on bioinformatic analyses for the individualized treatment of OV.

miRNAs are single-stranded, noncoding, small RNAs of approximately 22 nucleotides in length, generated from endogenous heparin loop miRNA precursors, which are enrolled in the regulation of gene expression at the transcriptional or posttranscriptional level (Kim, 2005; Graves and Zeng, 2012). Additionally, existing research recognizes the pivotal role of miRNAs in many biological behaviors of cancers, as penetratively analyzed in the previous studies, including angiogenesis, tissue invasion or metastasis, deregulating cell energetics, and avoiding immune destruction (Bueno et al., 2008; Nicoloso et al., 2009; Hanahan and Weinberg, 2011; Fei et al., 2012; Wei et al., 2013). In particular, the aberrant expression of some ovary-specific miRNAs can be a crucial putative biomarker or indicator in the diagnosis, individualized treatment, and prognosis of OV (Pal et al., 2015). For example, miR-182 was confirmed to be upregulated in OV cases using a public dataset from the Gene Expression Omnibus (GEO) database, and two hub genes, MCM3 and GINS2, were indicated to be associated with the worse overall survival of patients (Li and Li, 2019). Based on these predictive hallmarks, miRNAs involved in tumorigenesis and progression have aroused wide attention.

miRNA-1827 or miRNA-1827, as a novel miRNA, acts with multiple biological functions in various types of cancers. miRNA-1827 was initially identified in HeLa cells by miRDeep sequencing (Friedlander et al., 2008). A recent study demonstrated that it could tightly regulate the expression level and function of TP53 to suppress the oncogenesis of human colorectal cancer by targeting MDM2, which could bind to and degrade the P53 protein under ubiquitylation (Zhang et al., 2016). Furthermore, similar research in ulcerative colitis patients also indicated that miRNA-1827 in serum was decreased and was correlated with a higher risk of colorectal cancer (Polytarchou et al., 2015). Besides, miRNA-1827 was a putative therapeutic target in lung cancer in vitro and in vivo, and it could modulate the migration, angiogenesis, or invasion directly by targeting the CRKL molecule (Ho et al., 2014; Ho et al., 2018). However, as much as it has been reported to be downregulated in most types of cancers as a tumor repressor, very few studies have investigated its role in OV. Therefore, it will be essential to verify its clinical significance as a new intervention target and reveal the underlying mechanisms by screening out the possible targets of miRNA-1827 for further application.

This study assessed the influences of the differential expression of miRNA-1827, with all data collected from six GEO microarrays. To illuminate the potential modulatory mechanisms, the downstream regulators targeted by miRNA-1827 were predicted and verified by 12 prophetic databases together with the Gene Expression Profiling Interactive Analysis (GEPIA) database (the latter is based on the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEX) data). Subsequently, Gene Oncology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein–protein interaction (PPI) network analysis were comprehensively performed.

MATERIALS AND METHODS

Gene Expression Omnibus Microarray Acquisition

To retrieve the microarray data on miRNA-1827 in OV, we searched the GEO database (https://www.ncbi.nlm.nih.gov/geo/) with the keywords as listed: (("ovarian" OR "ovary" OR "ovaries") AND ("cancer" OR "cancerous" OR "carcinoma" OR "carcinomatous" OR "neoplasm" OR "neoplasms" OR "adenoma" OR "adenomas" OR "adenocarcinoma" OR "adenocarcinomas" OR "malignancy" OR "tumor" OR "tumors") AND ("micro RNA" OR "microRNA" OR "miRNA" OR "non-coding RNA" OR "ncRNA" OR "small RNA")). The retrieval expression was filtered by “Homo sapiens” of the organism. In addition, the inclusion criteria were set as follows: 1) patients diagnosed with OV and its subtypes were included; 2) the experiments were performed with cancerous samples and normal control (NC), and for at least three, biological
duplications were investigated; 3) the bio-specimens were isolated from tissue, serum, or urine; and 4) the expression profiling data of miRNA-1827 were available. All data were processed according to the flow diagram, as shown in Figure 1.

**Statistical Comparison and Comprehensive Meta-Analysis for Microarrays**

The expression profiling results of miRNA-1827 derived from the queried microarrays were aggregated to calculate the number, mean value (M), and standard deviation (SD) for comparisons between the two groups by the Student’s t-test, and the expressional results were displayed using the ggpubr package. Then, a comprehensive meta-analysis by the meta package was performed to evaluate the data from all selected microarrays, for which the expression of miRNA-1827 between groups was illustrated by the forest plot that displayed the standardized mean difference (SMD) and the 95% confidential interval. It should be mentioned that the DerSimonian–Laird estimator for $\tau^2$ and $I^2$ statistics was applied to determine whether to adopt a random-effects model or fixed-effects model for the pooled estimate. The sensitivity analysis was conducted to assess the heterogeneity among different studies, and if it did exist, the subgroup analysis was carried out to ascertain the reason for the heterogeneity. To measure publication bias, Begg’s test and funnel plots were used.

**Roles of miRNA-1827 in the Proliferation of Ovarian Cancer Cells**

To verify the expression level of miRNA-1827 in OV cells, five types of OV cell lines (OV1063, Caov3, SKOV3, OVCAR3, and A2780 cells) and two kinds of human normal ovarian epithelial cells (IOSE80 and HOSEpiC) were used for further experiments. As directed, these cells were cultured in the recommended medium in a humidified atmosphere supplemented with 5% CO$_2$. Cells were passaged by trypsin digestion at 80% confluence. To quantify the content of miRNA-1827, the total miRNAs were extracted by an miRNA Isolation Kit (Vazyme, China). The purified miRNAs were then pretreated with DNase I

---

**FIGURE 1** | Flow diagram of the research design. This flowchart presented a comprehensive meta-analysis to validate the expression among different microarrays in OV, and bioinformatic analysis was performed to investigate the putative target genes. Hub genes were filtered out for further research on their prognostic significance.
To locate the targets for miRNA-1827 in Ovarian Cancer

Putative Targets of miRNA-1827 in Ovarian Cancer

To validate the expression patterns of the hub genes, gene expression profiles of OV samples were downloaded from the project of the International Cancer Genome Consortium (ICGC) database (https://dcc.icgc.org/) (Zhang et al., 2019). Besides, data

Functional Enrichment Analysis for Latent Target Genes

GO term enrichment and KEGG analyses have been widely applied to consolidate biology in compiling a disciplined, structured, and defined glossary for numerous genes. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) (Dennis et al., 2003) was utilized to associate functional terms with the uploaded genes using the clustering algorithms. The enriched results were classified into the biological process, cellular component, and molecular function terms, as revealed by the bubble plots, and integrated functional pathways, as shown in the chordal graph by ggplot2 and GOplot packages.

Identification and Characteristics of Prognostic Hub Genes

To identify the hub genes that interacted with miRNA-1827 and were relevant to the clinical prognosis, the latent target genes described above were explored by the PPI analysis via the online database STRING (https://string-db.org/cgi/input.pl) (von Mering et al., 2003). The outcomes were presented by Cytoscape software (https://cytoscape.org/) (Shannon et al., 2003). On this basis, gene modules or clusters were filtered via the MCODE plugin with a K-core value > 2. Then, four clusters were screened out, and 34 genes were identified for subsequent bioinformatic analyses. Among these genes, two hub genes, SPTBN2 and BCL2L1, were matched with prognostic significance and validated by the GEPIA survival and correlation analyses.

Validation of the Expression Patterns of the Hub Genes

To validate the expression patterns of the hub genes, gene expression profiles of OV samples were downloaded from the project of the International Cancer Genome Consortium (ICGC) database (https://dcc.icgc.org/) (Zhang et al., 2019). Besides, data

TABLE 1 | Gene Expression Omnibus datasets involved.

| Accession | GPL | Year | Ovarian cancer | Normal control | Source |
|-----------|-----|------|---------------|----------------|--------|
| GSE119056 | GPL21572 | 2019 | 6 | 3.89 | 0.59 | 3 | 11.01 | 9.18 | Tissue |
| GSE83693 | GPL22079 | 2017 | 16 | 0.27 | 0.16 | 4 | 1.10 | 0.07 | Tissue |
| GSE53829 | GPL18138 | 2014 | 45 | 30.99 | 2.05 | 14 | 31.26 | 2.35 | Tissue |
| GSE47841 | GPL14813 | 2014 | 21 | 2.20 | 0.52 | 9 | 3.00 | 0.93 | Tissue |
| GSE48885 | GPL14843 | 2014 | 5 | –1.82 | 0.72 | 5 | –1.43 | 0.63 | Serum |
| GSE58617 | GPL18402 | 2015 | 5 | –5.73 | 1.78 | 5 | –5.92 | 7.64 | Urine |

M, mean; SD, standard deviation.
generated from normal tissues were obtained from the GEPIA database as controls. All the data after normalization were processed to identify the expression difference of \textit{SPTBN2} and \textit{BCL2L1} between groups. In order to better understand these two hub genes, the Human Protein Atlas (HPA) database (http://www.proteinatlas.org/) (Uhlen et al., 2015) was further utilized to exploit the relevant information on the location and protein expression in the cancer specimens compared with controls through the cell, tissue, and pathology atlas modules of this tool for further qualitative analyses.

### The Tumor Immunoregulatory Network Coordinated by the Hub Genes

To determine the potential mechanisms of the hub genes in the immunoregulatory system in OV, we explored the Tumor Immune Estimation Resource (TIMER) online tool (https://cistrome.shinyapps.io/timer/) (Li et al., 2017). We investigated the correlation between the hub gene expression and abundance of immune infiltration, and the association between clinical outcome and abundance of immune infiltration. The estimate algorithm was applied to determine the immune–stromal component in the tumor microenvironment (TME) of each sample utilizing the estimate package in R software (https://r-forge.r-project.org/). The limma package was applied to normalize the gene expression profile from the TCGA database to evaluate the proportion of tumor-infiltrating immune cells (TICs), and then a standardized gene expression profile was uploaded to CIBERSORT (Newman et al., 2015). The deconvolution algorithm was introduced here to estimate the TIC abundance. According to the average abundance of CD4$^+$ T cells or dendritic cells, Kaplan–Meier analysis by the log-rank test analysis was

![Figure 2](image-url)
carried out to assess survival possibility with the differential expression of \textit{SPTBN2} and \textit{BCL2L1}.

\section*{RESULTS}

\subsection*{Confirmation of the Expression of \textit{miRNA-1827} and the Meta-Analysis Results}

There were six microarrays included in this study from the GEO database, as depicted in Table 1. In total, four datasets were collected from tissue samples (GSE119056, GSE83693, GSE53829, and GSE47841), and the rest were from either serum (GSE48485) or urine specimens (GSE58517). Concerning the expression data from those datasets of \textit{miRNA-1827}, this miRNA was downregulated in the OV group compared with the NC group in all enrolled datasets, and two of them were expressed with significance, including GSE83693 ($p = 1E-08$) and GSE47841 ($p = 0.036$), as shown in Figure 2. On this basis, a comprehensive meta-analysis was performed to precisely quantify the expression of \textit{miRNA-1827}. The calculation results are displayed in Figure 3A, for which the random-effects model was applied considering the existing heterogeneity ($I^2 = 78\%$, $\tau^2 = 1.1064$, $p < 0.01$). Results also indicated that \textit{miRNA-1827} was remarkably downregulated in the OV group (SMD = $-1.22$ (95\% CI: $-2.21$; $-0.23$), with a degree of heterogeneity ($I^2 = 78\%$, $\tau^2 = 1.1064$, $p < 0.01$).

To clarify the source of heterogeneity, subgroup analysis was conducted according to the source types, divided into two groups (the non-tissue group or tissue group), as depicted in Figure 4. The results demonstrated that no heterogeneity was observed in the non-tissue group (SMD = $-0.57$ (95\% CI: $-1.47$; $-0.34$), $Q = 0.01$, $\tau^2 = 0$, $\Gamma = 0.0\%$). Conversely, the tissue group was found to
account for the heterogeneity (SMD = −1.67, 95% CI [−3.18, −0.17], Q = 22.90, $t^2 = 1.8946$, $I^2 = 87\%$). In this group, the study (GSE53829) might be the main cause with the largest number of samples included in the microarrays (45 from the OV samples and 14 from the NC samples).

Subsequently, we explored the expression of miRNA-1827 in OV cells, and our results indicated that these cell lines exhibited consistent trends of declined expression in these five kinds of OV cells compared with the normal ovarian epithelial cells, as shown in Supplementary Figure S1A. Notably, there existed significant differences of miRNA-1827 in A2780 cells. Similarly, the expression level of miRNA-1827 in OVCAR3 cells differed from that of miRNA-1827 in HOSEpiC cells. According to these results, two cells, A2780 and OV1063, were chosen to be transfected with the mimic or inhibitor of miRNA-1827, respectively. A CCK8 assay was performed after transfection to determine the cell viability, and we found that the proliferation of A2780 cells was dramatically inhibited after being treated with the miRNA-1827 mimic ($p < 0.01$). However, the competitive inhibition of miRNA-1827 seemed not to make a meaningful difference, which might be reasonably explained by its relatively low content in OV cells (Supplementary Figure S1B).

**Functional Enrichment Results of the Target Genes and Their Core Modules**

There were 334 overlapped target genes selected as interactive genes with miRNA-1827, which were collected from 4,166 genes from the miRWALK database based on 12 servers and 2,611 overexpressed genes from the GEPIA database (Figure 5A). After the intersection, these 334 target genes were processed by bioinformatic analysis, and the results were interpreted by GO functional annotation and KEGG analysis in the DAVID database. For GO analysis, it was performed by three modules, including biological process, cellular component, and molecular function. For the biological process part, the extracellular matrix organization, cell–cell adhesion, regulation of cell proliferation and migration, actin skeleton organization, and mitochondrial membrane permeability were the main processes (Figure 5B, left panel). As for the cellular component, the cell–cell adherent junctions, extracellular exosome, transport vesicle, actin cytoskeleton, and lysosome were the primary components (Figure 5B, middle panel). Regarding the molecular function, the cadherin binding involved in cell–cell adhesion, myosin V binding, protein kinase binding, and protein self-association were the top enriched functions (Figure 5B, right panel). Concerning KEGG pathways, cell lung cancer, hepatitis C, tight junction, and cell adhesion molecules were the major involved pathways (Figure 5C). PPI network filtered out four main modules according to their MCODE score (Figure 6). These four clusters consisted of 34 nodes or genes, as listed in Table 2. As seen, GO and KEGG enrichment of these 34 genes demonstrated that they were mainly involved in the activation of caspase activity by cytochrome c, cell cycle, and regulation of mitochondria membrane permeability for the biological process (Figure 7A). The chromosome, cytosol, and kinetochore were enriched for the cellular component (Figure 7B), and the protein heterodimerization activity, purine ribonucleotide binding, and ATP binding for molecular function were determined (Figure 7C). The pathways in cancers, tight junction, and cell adhesion molecules for KEGG were identified (Figure 7D). All the valuable information above gave us some tips and clues of the localization and molecular functions of these genes in OV, which could help us narrow the targets down to several specific hub genes.

**Characteristics and Clinical Value of the Hub Genes**

According to the clinical value retrieved in the GEPIA and HPA databases in OV, two hub genes, SPTBN2 and BCL2L1, caught our attention. SPTBN2 and BCL2L1 were located in the cytosol or
cell junctions (SPTN2, Figure 7E, left) and mitochondria (BCL2L1, Figure 7E, right), respectively. Also, SPTN2 and BCL2L1 were negatively expressed in normal ovarian tissues at the protein level, as depicted by the immunohistochemical results (Figure 7F). As we could see, SPTN2 and BCL2L1 were overexpressed significantly in the OV group compared with the normal controls ($p < 0.05$), as shown in Figure 8A. The elimination of the inhibitory effect of miRNAs might account for the changes with the downregulation of miRNA-1827. To further verify the accuracy of these results, the gene expression profiles from the ICGC database were retrieved and processed. Consistent with the above findings, the contents of SPTN2 and BCL2L1 were relatively overexpressed in these 111 cases of OV patients compared with the controls ($p < 0.01$) (Supplementary Figures 2A,B). In addition, there existed a moderate correlation between the expression of SPTN2 and BCL2L1 with a significant difference ($p = 9.6E-59$, Figure 8B). Furthermore, OV patients in stage II, III, or IV shared similar gene expression patterns for SPTN2 (Figure 8C) or BCL2L1 (Figure 8D) in cancer tissues. However, the upregulation of the two hub genes would lead to an elevated risk of death, as displayed in the survival curves (Figure 8E) (SPTN2, upper panel, log-rank $p = 0.012$, HR = 1.4; BCL2L1, lower panel, log-rank $p = 0.034$, HR = 1.3). To further investigate the hub genes and their crucial roles in various types of cancers, we detected the gene and protein expression levels among different cancers. The images for qualitative analysis in this study were obtained from the HPA database. The results demonstrated that SPTN2 and BCL2L1 were relatively overexpressed in most cancers compared with normal tissues, as delineated in Figure 9A at the gene level and Figure 9B at the protein level. These results indicated that
the two hub genes might be suitable biomarkers not only inOV but also in other cancers, such as breast cancer, lung cancer, colon cancer, and prostate cancer.

The Potential Mechanisms of the Hub Genes Involved in Tumor Immunomodulatory Actions

To further decipher the function of the hub genes in the modulation of the tumor-infiltrating immune cells, the TIMER database was used to determine the changes of immunocytes in OV tissue and their interactive relationship with the hub genes. SPTBN2 was negatively correlated, to a certain degree, with the infiltration of CD4+ T cells (Figure 10Ai, partial.cor = −0.119, \( p = 9.10\times10^{-03} \)) as well as dendritic cells (Figure 10Aii, partial.cor = −0.118, \( p = 9.53\times10^{-03} \)). Similarly, BCL2L1 was also negatively correlated, up to a point, with the infiltration of dendritic cells (Figure 10Aiii, partial.cor = −0.158, \( p = 5.17\times10^{-04} \)). All those results suggested that the two hub genes might play a crucial role in adjusting CD4+ T cells and dendritic cells in the OV microenvironment. The higher the expression of the hub genes, the lower the infiltration of the immunocytes. Lower immunocyte infiltration of CD4+ T cells and dendritic cells meant poor prognosis as presented in Figure 10B. At the same time, we also explored the survival probability with the differential expression of SPTBN2 and BCL2L1 with the alteration of TIC abundance (CD4+ T cells and dendritic cells), as shown in Supplementary Figure S3. Our results demonstrated that the
application of bioinformatics and computational biology for information mining to determine the expression level of miRNA-1827. As reported, miRNA-1827 is downregulated in most cancer samples, such as human lung adenocarcinoma cells and lymphoblastic leukemia clinical cases or cell lines, and the decreased level is correlated with tumor grade and lymph node metastasis (Guo et al., 2020; Naderi et al., 2020). In line with these findings, our research has also observed the reduced expression of miRNA-1827 in all OV cases. The significantly differential expression was further confirmed by the comprehensive meta-analysis and qRT-PCR detection of seven types of OV cell lines or normal ovarian epithelial cells. Furthermore, a significant difference was noticed in the proliferation of OV cells after transfection of the miRNA-1827 mimic. As previously described, miRNA-1827 has been proved to suppress the development of lung adenocarcinoma by targeting oncogenic genes MYC and FAM83F (Fan et al., 2020). Another study also confirmed that a single-nucleotide polymorphism within the binding site of miRNA-1827 on the 3’ UTR of MYCL1 could repress the expression level of the latter, triggering the progression of small-cell lung cancer (Xiong et al., 2011). These results signified that the overexpression of miRNA-1827 could also act as a potential intervention pattern of OV. Subsequently, 334 candidate interactive genes targeted by miRNA-1827 were screened out based on the miRWALK and GEPIA databases; GO and KEGG enrichment analyses were carried out to determine the characteristics and functions of these genes. According to the results, the most meaningful finding that emerged from the analyses was that these targets of miRNAs were involved in the regulation of cell proliferation and migration, extracellular matrix organization, cell–cell adhesion, actin skeleton organization, and actin filament bundle assembly. Intriguingly, miRNA-1827 has been known to engage in biological activities chiefly, including the modulation of cellular proliferation, invasion, and metastasis in reproductive or nonproductive tumors (Wang et al., 2020; Shen et al., 2021). It is worth noting that altered cytoskeleton-associated proteins have been proven to produce an effect on the clinical outcomes of OV patients during malignant progression (Schiewek et al., 2018). In addition to this, cytoskeletal dynamics, such as those of the actin filament, play a pivotal role in enhanced cell motility to promote OV metastasis through

### DISCUSSION

With the advent of high-throughput sequencing and microarray technologies, a striking upsurge in identifying novel biomarkers and the construction of molecular networks in various diseases is in the making based on the advances of bioinformatic analyses (Udhyaa Kumar et al., 2020b; Mishra et al., 2021). At the same time, a growing body of evidence from gene expression profiles and microarrays has confirmed the significance of miRNAs in the tumorigenesis and progression of various types of cancers (Rupaimoole and Slack, 2017). The comparatively deep explorations on their roles have promoted the identification of novel biomarkers and application of these potential intervention targets toward the era of miRNA therapeutics (Hayes et al., 2014).

Previous studies have revealed that miRNA-1827 functions as a tumor suppressor in various types of cancers since it is involved in tumor proliferation, invasion, migration, and angiogenesis (Ho et al., 2018). However, to date, no tangible proof has ever uncovered the relationship between miRNA-1827 and its clinical significance in OV. Here, we performed systemic bioinformatic analyses to determine its pivotal role in OV and its intrinsic targets. The highlights of this study are the inclusion of a larger sample size by pooling similar studies and the

### TABLE 2 | 34 target genes from the four clusters.

| Names  | Clusters | MCODE score | Names  | Clusters | MCODE score | Names  | Clusters | MCODE score |
|--------|----------|-------------|--------|----------|-------------|--------|----------|-------------|
| NCAAG  | One      | 11.1        | TMED10 | Two      | 5.6         | CXCL10 | Four     | 4.6         |
| POLQ   | One      | 11.1        | KDELRE3| Two      | 5.6         | OAAS3  | Four     | 4.6         |
| ARHGAP11A | One   | 11.1       | KIF1A  | Two      | 5.6         | rRF1   | Four     | 4.6         |
| S2D5    | One      | 11.1        | ARF53  | Two      | 5.6         | RASAD2 | Four     | 4.6         |
| BUB1B   | One      | 11.1        | COPZ1  | Two      | 5.6         | rFT2   | Four     | 4.6         |
| MKI67   | One      | 11.1        | SPTBN2 | Two      | 5.6         | TP5    | Four     | 4.6         |
| CDKN3  | One      | 11.1        | RNF144B| Three    | 5.0         | CAPN1  | Four     | 4.6         |
| STIL    | One      | 11.1        | TCEB2  | Three    | 5.0         | H2AFX  | Four     | 4.6         |
| DTL     | One      | 11.1        | DTX3L  | Three    | 5.0         | BAK1   | Four     | 4.6         |
| HJURP   | One      | 11.1        | CCNF   | Three    | 5.0         | BCL2L1 | Four     | 4.6         |
| TOP2A   | One      | 11.1        | FBXW9  | Three    | 5.0         | CYCS   | Four     | 4.6         |
| DEPDC1  | One      | 11.1        |        |          |             |        |          |             |
epithelial–mesenchymal transition (Lee et al., 2013). To elucidate the hub interactions among PPI networks, the MCODE plugin in Cytoscape software was applied, and finally, 34 node genes were identified. These genes were the main components of the chromosome (centromeric region), kinetochore, organelle lumen, and replication fork. As expected, these genes exerted considerable influence on the cell cycle process, chromosome segregation, DNA packaging, activation of caspase activity, and cell death. Based on this, two hub genes, SPTBN2 and BCL2L1, were determined as target genes of miRNA-1827 for further research according to the clinical value retrieved in the GEPIA and HPA databases in OV. SPTBN2 is a protein-encoding gene, and its protein product belongs to the spectrin family. SPTBN2 was previously reported in the development of cognition and motion or neurologic disorders (Forman et al., 2012; Lise et al., 2012). Additionally, it was recently identified as a signature gene, emerging in seven common cancers in the pathogenesis of cancers and pattern recognition (Wen et al., 2018). In the present study, SPTBN2 was confirmed for the first time to be upregulated in OV at gene and protein levels. Most studies mainly focused on the role of SPTBN2 in neurogenesis and degenerative diseases as a structural carrier for the stabilization and activation of membrane channels, receptors, and transporters (Yildiz Bolukbasi et al., 2017; Louis and Faust, 2020). In terms of cancer research, it was identified to be associated with the tumor progression and survival of colorectal cancer patients with m6A modifications (Zhang et al., 2021). Besides, it has also been reported to be a member of a novel, competing, endogenous RNA network for the prognosis of bladder cancer (Zhang et al., 2021). As for BCL2L1, it is an anti-apoptotic regulator of the BCL-2 family and causes momentous effects on the integrity of the mitochondria membrane, autophagy, and cell survival.

FIGURE 7 | GO analysis for 34 genes from four selected modules and characteristics of the promising hub genes, SPTBN2 and BCL2L1. (A) Biological process for the 34 genes. (B) Cellular components for the 34 genes. (C) Molecular function for the 34 genes. (D) KEGG pathways enriched for the 34 genes. (E) Cellular localization from the cell atlas of the HPA database, with SPTNB2 localizing to the cytosol and cell junctions, stained in A-431 and MCF-7 cell lines (left), and BCL2L1 localizing to mitochondria, stained in MCF-7 cell line (right). (F) Decreased expression of SPTBN2 (left) and BCL2L1 (right) in normal ovarian tissue.
BCL2L1 has made significant differences in tumor targeting therapy, as a novel tumor promoter, for hepatocellular carcinoma, lung cancer, gastric cancer, and cervical cancer (Park et al., 2015; Cai et al., 2017). Here, we found that BCL2L1 was also overexpressed in OV specimens. As a pro-oncogene, the aberrant expression of BCL2L1 is related to a shortened disease-free survival, which accrues in patients with recurrence following chemotherapeutic interventions in OV (Williams et al., 2005). Preclinical research has also demonstrated that the ectopic expression of BCL2L1 confers resistance to various pharmaceutical products, such as cisplatin, vincristine, and gemcitabine (Schniewind et al., 2004). Posttranslational modification by deamidation of BCL2L1 has also been proven to be implicated in drug resistance to DNA-damaging medicaments (Deverman et al., 2002), and the pro-survival action of the oncogenic tyrosine kinase LCK following DNA damage may be mediated partly by restraining deamidation of BCL2L1 as well (Zhao et al., 2004). All these findings have therefore specified the promising therapy of targeting BCL2L1 in OV management. Besides, our investigation showed that these two hub genes were co-expressed and correlated with each other. The overexpression of these molecules would increase the risk of death or give rise to a poorer prognosis. During the progression of OV, they were persistently highly expressed across stages II, III, and IV. It should be mentioned that SPTBN2 and BCL2L1 were upregulated together not only in OV but also in various types of cancers, which were validated by various types of tumors and normal samples at both the gene and protein levels. Overall, these findings have therefore specified the promising therapy of targeting these genes in OV management.

(Feng et al., 2019). BCL2L1 has made significant differences in tumor targeting therapy, as a novel tumor promoter, for hepatocellular carcinoma, lung cancer, gastric cancer, and cervical cancer (Park et al., 2015; Cai et al., 2017). Here, we found that BCL2L1 was also overexpressed in OV specimens. As a pro-oncogene, the aberrant expression of BCL2L1 is related to a shortened disease-free survival, which accrues in patients with recurrence following chemotherapeutic interventions in OV (Williams et al., 2005). Preclinical research has also demonstrated that the ectopic expression of BCL2L1 confers resistance to various pharmaceutical products, such as cisplatin, vincristine, and gemcitabine (Schniewind et al., 2004). Posttranslational modification by deamidation of BCL2L1 has also been proven to be implicated in drug resistance to DNA-damaging medicaments (Deverman et al., 2002), and the pro-survival action of the oncogenic tyrosine kinase LCK following DNA damage may be mediated partly by restraining deamidation of BCL2L1 as well (Zhao et al., 2004). All these findings have therefore specified the promising therapy of targeting BCL2L1 in OV management. Besides, our investigation showed that these two hub genes were co-expressed and correlated with each other. The overexpression of these molecules would increase the risk of death or give rise to a poorer prognosis. During the progression of OV, they were persistently highly expressed across stages II, III, and IV. It should be mentioned that SPTBN2 and BCL2L1 were upregulated together not only in OV but also in various types of cancers, which were validated by various types of tumors and normal samples at both the gene and protein levels. Overall, these findings have therefore specified the promising therapy of targeting these genes in OV management.

(Figure 8) Clinical value of the two hub genes. (A) Expressional levels between OV and NC groups for SPTBN2 (left) and BCL2L1 (right) (n = 426 for OV or 88 for NC, p < 0.05). (B) The correlation analysis for SPTBN2 and BCL2L1 (r = 9.6E-59, R = 0.63). (C) The overall survival for patients with high or low SPTBN2 (upper panel, log-rank p = 0.012, HR = 1.4) and with high or low BCL2L1 (upper panel, log-rank p = 0.034, HR = 1.3). (D) Expression of SPTBN2 with different stages in OV (F = 1.16, Pr(F) = 0.316). (E) Expression of BCL2L1 with different stages in OV (F = 0.354, Pr(F) = 0.702).
discoveries suggested the prognostic significance of SPTBN2 and BCL2L1 as therapeutic biomarkers targeted by miRNA-1827 in OV.

In the following exploration, we have focused on the roles of SPTBN2 and BCL2L1 in the tumor microenvironment from the perspective of immune modulation by retrieving data from the TIMER database. Existing studies demonstrate that the interaction between tumor cells and host cells is indispensable for the oncogenesis and progression of tumors (Grivennikov et al., 2010). Within this environment and upon tumor-driven stimuli, tumors can form a tumor-permissive soil with reprogrammed host cells that exhibit tumor-supporting phenotypes (Binnewies et al., 2018). Intriguingly, this study revealed that SPTBN2 and BCL2L1 could generate adverse effects on the decreased infiltration of CD4+ T cells and dendritic cells in OV, which seemed likely to bring about a more unsatisfactory prognostic outcome. The reduced abundance of CD4+ T cells and dendritic cells in this study was

FIGURE 9 | Differential expression of the two hub genes in various types of tumors compared with NC. (A) Distributions of gene expression levels are displayed using box plots from the TIMER database by the Wilcoxon test for SPTBN2 (upper panel) and BCL2L1 (lower panel). (B) Differential expression at protein levels between cancers and normal tissues, verified by the protein atlas in the HPA database including the breast, lung, colon, and prostate.
confirmed to be associated with declined cumulative survival rate. In order to seek the reasons for the reduction of CD4⁺ T cells and dendritic cells mediated by SPTBN2 or BCL2L1, the related chemokine CCL27 and the receptor CCR10 were explored. In terms of the traits of the two molecules, CCL27, as a member of the chemokine ligand family, would participate in the homing of immunocytes by specifically binding to CCR10 (Fujita et al., 2006). CCL27 was once regarded as a sensitive serum biomarker to distinguish nasopharyngeal carcinoma patients from healthy donors (highly expressed in abnormal populations due to the activated immune system) (Mao et al., 2018). As reported previously, CCL27 could inhibit tumor growth to a certain extent through recruiting natural killer cells or T cells locally (Gao et al., 2003) and could intensify the resistance to tumor formation as well (Okada et al., 2004). In addition, CCL27 could enter into a chemotaxis role for the recruitment and activation of CD4⁺ T cells. Our study found that these molecules were also negatively influenced by SPTBN2 or BCL2L1. In accordance with our studies, people once reported (Khaioullina et al., 2015) that the reduced chemokine of CCL27 could help cancers to evade immunological surveillance, thereby resulting in immune escape (Fivars et al., 2007). The results above delineate the alteration of SPTBN2 and BCL2L1 in view of tumor immune modulation mediated by CCL27 and CCR10, resulting in a reduced abundance of CD4⁺ T cells and dendritic cells.

The limitations of the current study need to be pointed out: the mutual interactions between miRNA-1827 and its two hub genes should be further validated to ensure the target-binding action. Besides, the effects of miRNA-1827 in OV should be verified in animal models to promote its clinical application.

CONCLUSION
In conclusion, the present study takes advantage of retrieved data from microarrays or gene expression profiles and integrative bioinformatic analyses. Therefore, we have identified that miRNA-1827 as a novel biomarker is associated with the proliferation and prognosis of OV by targeting SPTBN2 and BCL2L1. These hub genes are determined to be
involved in the tumor immunomodulatory process mediated by CCL27 and CCR10. On the whole, miRNA-1827 from computational biology analysis exerts promising effects as an intervention target in OV management in future applications.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GSE119056, GSE83693, GSE53829, GSE47841, GSE48485, and GSE58517.

AUTHOR CONTRIBUTIONS

PHF, ZTG, ZXG, and LL: collection and assembly of data, data analysis, and manuscript writing. QY: conception and design, administrative support, and final approval of the manuscript.

REFERENCES

Allemanni, C., Matsuda, T., Di Carlo, V., Harewood, R., Matz, M., Nikić, M., et al. (2018). Global Surveillance of Trends in Cancer Survival 2000–14 (CONCORD-3): Analysis of Individual Records for 37 513 025 Patients Diagnosed with One of 18 Cancers From 322 Population-Based Registries in 71 Countries. Lancet 391 (10125), 1023–1075. doi:10.1016/S0140-6736(17)33323-6

Binnewies, M., Roberts, E. W., Kersten, K., Chan, V., Fearon, D. F., Merad, M., et al. (2018). Understanding the Tumor Immune Microenvironment (TIME) for Effective Therapy. Nat. Med. 24 (5), 541–550. doi:10.1038/s41591-018-0014-x

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global Cancer Statistics 2018: Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 68 (6), 394–424. doi:10.3332/caac.21492

Bueno, M. J., de Castro, I. P., and Malumbres, M. (2008). Control of Cell Division Pathways by microRNAs. Cell Cycle 7 (20), 3143–3148. doi:10.4161/cc.7.20.6833

Busk, P. K. (2014). A Tool for Design of Primers for microRNA-Specific Quantitative RT-qPCR. BMC Bioinformatics 15, 29. doi:10.1186/1471-2105-15-29

Cai, J., Liu, T., Jiang, X., Guo, C., Liu, A., and Xiao, X. (2017). Downregulation of USP18 Inhibits Growth and Induces Apoptosis in Hepatitis B Virus-Related Hepatocellular Carcinoma Cells by Suppressing BCL2L1. Exp. Cell Res. 358 (2), 315–322. doi:10.1016/j.yexcr.2017.07.006

Dennis, G., Jr., Sherman, B. T., Hosack, D. A., Yang, J., Gao, W., Lane, H. C., et al. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 4 (5), P3. doi:10.1186/gb-2003-4-9-9

Deverman, B. E., Cook, B. L., Manson, S. R., Niederhoff, R. A., Langer, E. M., Rosová, I., et al. (2002). Bcl-xL Deamidation is a Critical Switch in the Regulation of the Response to DNA Damage. Cell 111 (1), 51–62. doi:10.1016/S0022-8050(02)00972-8

Duan, S., Yu, S., Yuan, T., Yao, S., and Zhang, L. (2019). Exogenous Let-7a-5p Induces A549 Lung Cancer Cell Death through BCL2L1-Mediated PI3K/Akt Signaling Pathway. Front. Oncol. 9, 808. doi:10.3389/fonc.2019.00808

Fan, G., Xu, P., and Tu, P. (2020). MiR-1827 Functions as a Tumor Suppressor in Lung Adenocarcinoma by Targeting MYC and FAM83F. J. Cell Biochem. 121 (2), 1675–1689. doi:10.1002/jcb.29402

Fei, X., Qi, M., Wu, B., Song, Y., Wang, Y., and Li, T. (2012). MicroRNA-195-5p Suppresses Glucose Uptake and Proliferation of Human Bladder Cancer T24 Cells by Regulating GLUT1 Expression. FEBs Lett. 586 (4), 392–397. doi:10.1016/j.febslet.2012.01.006

FUNDING

This study was funded by the Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (grant number 2020-PT320-003).

ACKNOWLEDGMENTS

We acknowledge GEO, GTEx, and TCGA databases for providing their platforms and contributors for uploading their meaningful datasets. The authors would like to thank Editage (www.editage.cn) for English language polishing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2021.687576/full#supplementary-material

Forman, O. P., De Risio, L., Stewart, J., Mellersh, C. S., and Beltran, E. (2012). Genome-Wide miRNA Sequencing of a Single Canine Cerebellar Cortical Degeneration Case Leads to the Identification of a Disease Associated SPTBN2 Mutation. BMC Genet. 13, 55. doi:10.1186/1471-2156-13-55

Friedländer, M. R., Chen, W., Adamidi, C., Maaskola, J., Einspanier, R., Knespel, S., et al. (2008). Discovering microRNAs From Deep Sequencing Data Using miRDeep. Nat. Biotechnol. 26 (4), 407–415. doi:10.1038/nbt1394

Fu, D., Zhang, B., Yang, L., Huang, S., and Xin, W. (2020). Development of an Immune-Related Risk Signature for Predicting Prognosis in Lung Squamous Cell Carcinoma. Front. Genet. 11, 978. doi:10.3389/fgene.2020.00978

Fujita, Y., Abe, R., Sasaki, M., Honda, A., Furuichi, M., Asano, Y., et al. (2006). Presence of Circulating CCR10+ T Cells and Elevated Serum CTACK/CCL27 in the Early Stage of Mycosis Fungoides. Clin. Cancer Res. 12 (9), 2670–2675. doi:10.1158/1078-0432.CCR-05-1513

Gao, J. Q., Tsuda, Y., Katayama, K., Nakayama, T., Hatanaka, Y., Tani, Y., et al. (2003). Antitumor Effect by Interleukin-11 Receptor Alpha-locus chemokine/ CCL27, Introduced Into Tumor Cells through a Recombinant Adenovirus Vector. Cancer Res. 63 (15), 4420–4425

Graves, P., and Zeng, Y. (2012). Biogenesis of Mammalian microRNAs: A Global View. Genomics Proteomics Bioinformatics 10 (5), 239–245. doi:10.1002/gpb.2012.06.004

Griewank, S. I., Greten, F. R., and Karin, M. (2010). Immunity, Inflammation, and Cancer. Cell 140 (6), 883–899. doi:10.1016/j.cell.2010.01.025

Guo, X., Wang, Z., Sun, Q., Sun, C., Hua, H., and Huang, Q. (2020). The Inhibitory Effect of microRNA-1827 on Anoikis Resistance in Lung Adenocarcinoma A549 Cells via Targeting Caveolin-1. Acta Biochim. Biophys. Sin (Shanghai) 52 (10), 1148–1155. doi:10.1093/abbs/gmaa102

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of Cancer: The Next Generation. Cell 144 (5), 646–674. doi:10.1016/j.cell.2011.02.013

Hayes, J., Peruzzi, P. P., and Lawler, S. (2014). MicroRNAs in Cancer: Bionarkers, Functions and Therapy. Trends Mol. Med. 20 (8), 460–469. doi:10.1016/j.molmed.2014.06.005

Ho, C. S., Noor, S. M., and Nagoor, N. H. (2018). MiR-378 and MiR-1827 Regulate Tumor Invasion, Migration and Angiogenesis in Human Lung Adenocarcinoma by Targeting RBX1 and CRKL, Respectively. J. Cancer 9 (2), 331–345. doi:10.7150/jca.18188

Ho, C. S., Yap, S. H., Phuah, N. H., In, L. L. A., and Hasina, N. (2014). MicroRNAs Associated With Tumour Migration, Invasion and Angiogenic Properties in A549 and SK-Lu1 Human Lung Adenocarcinoma Cells. Lung Cancer 83 (2), 154–162. doi:10.1016/j.lungcan.2013.11.024

Khabboullina, S. F., Gumerova, A. R., Khafizova, I. F., Martynova, E. V., Lombardi, V. G., Bellusci, S., et al. (2015). CCL27: Novel Cytokine With Potential Role in
Yang, Y., Qi, S., Shi, C., Han, X., Yu, J., Zhang, L., et al. (2020). Identification of Metastasis and Prognosis-Associated Genes for Serous Ovarian Cancer. *Biosci. Rep.* 40 (6), BSR20194324. doi:10.1042/BSR20194324

Yildiz Bolukbasi, E., Afzal, M., Mumtaz, S., Ahmad, N., Malik, S., and Tolun, A. (2017). Progressive SCAR14 With Unclear Speech, Developmental Delay, Tremor, and Behavioral Problems Caused by a Homozygous Deletion of the SPTBN2 Pleckstrin Homology Domain. *Am. J. Med. Genet. A.* 173 (9), 2494–2499. doi:10.1002/ajmg.a.38332

Zhang, C., Liu, J., Tan, C., Yue, X., Zhao, Y., Peng, J., et al. (2016). microRNA-1827 Represses MDM2 to Positively Regulate Tumor Suppressor P53 and Suppress Tumorigenesis. *Oncotarget* 7 (8), 8783–8796. doi:10.18632/oncotarget.7088

Zheng, M. J., Li, X., Hu, Y. X., Dong, H., Gou, R., Nie, X., et al. (2019). Identification of Molecular Marker Associated With Ovarian Cancer Prognosis Using Bioinformatics Analysis and Experiments. *J. Cell Physiol.* 234 (7), 11023–11036. doi:10.1002/jcp.27926

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

*Copyright © 2021 Feng, Ge, Guo, Lin and Yu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.*