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MiR-575 in Exosomes of Vaginal Discharge Is Downregulated in Ovarian Cancer Patients

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Abstract

Background/Aim: Ovarian cancer is asymptomatic in its early stages, and often diagnosed at advanced stages, leading to a high recurrence rate. In recent years, exosomes have been shown to be useful for early-detection, prognosis prediction, and treatment of cancer. Although many studies of cancer-related exosomes using other bodily fluids have been reported, there are few studies examining vaginal discharge, but none related to ovarian cancer. In this study, we investigated a method for early-detection of ovarian cancer using vaginal discharge, which are physically close to the fallopian tubes, where ovarian cancer originates, and can be easily collected from outside the body.
Materials and Methods: Vaginal discharge was collected from 30 patients with ovarian cancer and 29 patients with benign gynecological diseases, and exosomal miRNAs were extracted. Samples from each group were submitted to miRNA microarray in order to examine miRNAs with significant differences in expression levels. We further narrowed down the list to four miRNAs based on literature and microarray data and examined the expression levels of miRNAs in the malignant and benign groups by RT-qPCR.
Results: MiR-575 expression was significantly decreased in the malignant group compared to the benign group (p=0.00861). qPCR results were analyzed for several patient characteristics and no significant differences were found.
Conclusion: This is the first study to investigate exosomal miRNAs in vaginal discharge of ovarian cancer. Exosomal miR-575 in vaginal discharge may be used as a biomarker for ovarian cancer.

Keywords: Ovarian cancer, exosome, vaginal discharge, miRNA, miR-575.

Introduction

Epithelial ovarian cancer is the second leading cause of death among gynecological cancers. A total of 310,000 people worldwide is affected and 210,000 people die from the disease annually. Early detection is difficult, and approximately 75% of cases are diagnosed at an advanced stage, thus making treatment difficult (1).

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CA125 is often used as a tumor marker for ovarian cancer, but it can also be elevated in benign diseases such as endometriosis, pregnancy, and liver disease, and is not a specific marker for ovarian cancer, so it has a high false-positive rate (2). In addition, CA125 is often normal in early-stage ovarian cancer, so it is difficult to use for early diagnosis (2). HE4 is said to have high specificity for ovarian cancer, and when combined with CA125 to calculate the ROMA score, it is useful for predicting the malignancy of ovarian cancer and is expected to detect early-stage ovarian cancer, but the data is not yet sufficient (2). In Japan, the use of ctDNA to assess the risk of recurrence of colorectal cancer is covered by insurance, and it has been reported that ctDNA may also be used to predict prognosis in ovarian cancer (3). However, because it is a biomarker that depends on tumor volume, it is difficult to use for early diagnosis.

Exosomes are nano-sized extracellular vesicles (30-150 nm) secreted by almost all cells and are secreted into various body fluids such as blood, saliva, cerebrospinal fluid, urine, breast milk, and tears, and they are recognized as important mediators of intercellular communication (4-9). Exosomes contain proteins, RNA, DNA, and lipids (10). Exosomes secreted from cancer cells may contain substances specific to cancer, and it is hoped that detecting these may lead to early-detection of cancer (10, 11). Exosomes are also involved in cancer metastasis, inflammatory response, and tissue repair (10). Exosomes are expected to be used as biomarkers for diagnosis and prognosis prediction, cell-free therapy (exosome therapy), drug delivery systems, and cancer vaccines (10, 12).

MiRNAs are small non-coding RNAs with a length of approximately 22 nucleotides that mainly bind to the 3' untranslated region (3' UTR) of target mRNAs to suppress gene expression (13). MiRNAs are secreted into extracellular fluids and transported to target cells via vesicles such as exosomes (13). Extracellular miRNAs function as chemical messengers that mediate intercellular communication (13).

According to Salvi *et al.*, the surface antigens of exosomes differ between serum and urine, thus suggesting that the origin of exosomes is different (14). Urinary exosome research has been frequently conducted in prostate cancer, and several biomarkers from serum exosomes have been reported (14, 15). We hypothesized that, like urine, vaginal exosomes have a different composition from serum and may provide new biomarkers for ovarian cancer, as they do for prostate cancer. Unlike other medical specialties, gynecology frequently involves pelvic examinations, allowing for the relatively easy collection of vaginal discharge, which are one of the readily accessible body fluids. In addition, the vagina is connected to the abdominal cavity through the uterus and fallopian tubes, and we thought that there is a high possibility that exosomes originating from ovarian cancer tumor cells could be obtained. This is the first study to examine exosomes in the vaginal discharge of ovarian cancer patients. Exosomes in vaginal discharge have been reported in endometriosis and cervical cancer, but not in ovarian cancer (16). We conducted this study to examine exosomes in the vaginal discharge of ovarian cancer patients and benign patients, with the idea that differences in the relative amounts of certain miRNAs may lead to a novel early-detection method.

Materials and Methods

Study population. Thirty patients with epithelial ovarian cancer and 30 patients with benign gynecological diseases who visited the Gynecology Department of the Kobe University Hospital from July 2022 to October 2023 participated in the study. The benign group consisted of 20 uterine leiomyomas, four adenomyosis, seven mature cystic teratomas, one functional cyst, two endometriotic cysts, and three endometrial polyps. Seven patients had more than one benign disease. The malignant group consisted of 17 high-grade serous carcinomas, two endometrioid carcinomas, eight clear cell carcinomas, one mucinous carcinoma, and one low-grade serous carcinoma. One case in the malignant group was suspected to be endometrioid or clear cell carcinoma, but the histological type could not be determined. In the malignant group, seven patients were early stage (International Federation of Gynecology and Obstetrics (FIGO) stage I or II) and 23 were advanced stage (FIGO stage III or IV). Four patients were BRCA positive, and 12

Table I. *Clinical characteristics of ovarian cancer patients and benign gynecological disease patients in the study.*

	Malignant (n=30)	Benign (n=30)
Age	36-80	21-74
Stage		
Early	7	-
Advanced	23	-
Histological types		
HGSC	17	-
Endometrioid	2	-
Clear cell	8	-
Mucinous	1	-
LGSC	1	-
Undetermined	1	-
BRCA status		
Positive	4	-
Negative	16	-
NA	10	-
HRD status		
Positive	12	-
Negative	8	-
NA	10	-

HGSC: High grade serous carcinoma; LGSC: low grade serous carcinoma; BRCA: breast cancer gene; HRD: homologous recombination deficiency; NA: not available.

were homologous recombination deficiency (HRD) positive. 10 patients had not been tested and their genetic status was unknown (Table I).

Vaginal discharge samples. Vaginal discharge was collected at the time of examination at the Kobe University Hospital by wiping the inside of the vagina with swabs. The collected swabs were soaked in 4 ml of phosphate-buffered saline (PBS) (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The solution was collected, including the liquid squeezed from the swab. They were centrifuged at 3,000 × *g* for 15 min at 4˚C, and the supernatant was collected. Subsequently, the samples were then centrifuged at 12,000 × *g* for 15 min at 4˚C and the supernatant was collected (Figure 1). The centrifuged samples were stored at –80˚C until further use. If centrifugation was not possible immediately after collection, the collection tubes containing the soaked swabs were stored at 4˚C. In all cases, centrifugation was performed within one week.

Exosome extraction. Exosomes were extracted using the SmartSEC HT EV Isolation System (System Biosciences, Palo Alto, CA, USA) according to the manufacturer's protocol (Figure 1). The extracted exosomes were stored at –80˚C. Exosomal protein concentration was measured using Qubit 3.0 Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Exosomal miRNA microarray. MiRNA microarrays using 3D-Gene® Human miRNA Oligo chip (Toray Industries, Inc., Tokyo, Japan) was performed and analyzed by Kamakura Techno–Science, Inc. (Kanagawa, Japan). A total of four vaginal discharge samples were examined, two each from both the malignant and benign samples. The chip is a high-performance DNA chip substrate on which antisense oligonucleotides that detect human miRNAs selected from the miRBase database are spotted. Using 3D-Gene® miRNA labeling kit, miRNAs extracted from exosomes were labeled with green fluorescent dye, and the standard sample was labeled with red fluorescent dye. Hybridization reaction was then performed with the DNA chip. After the reaction, the washed chip was read with a 3D-Gene® Scanner 3000 (Toray Industries) to detect the signal. The analysis software used was GeneSpring GX (Agilent Technologies, Santa Clara, CA, USA). Each value was used as the global normalization when the signal intensity was corrected to a median of 25. A Log2 value when the 75 percentile was corrected to 1 was used as the Log2 (75^{th} percentile normalization), and the difference in the averages between the benign and malignant groups was used as the Log2 ratio. This was converted to an antilogarithmic number to use as a ratio. A ratio of global normalization of 0.5 or less was considered significant for downregulation, and 2 or more for upregulation. The raw data are available in the Gene Expression Omnibus database (GSE271256).

MiRNA extraction. MiRNAs were extracted using the QIAzol Lysis Reagent (QIAgen, Hilden, Germany) and miRNeasy Mini Kit (QIAgen) according to the protocol. An miRNA quality check was performed using a Bioanalyzer 2100 (Agilent Technologies). Total RNA obtained from

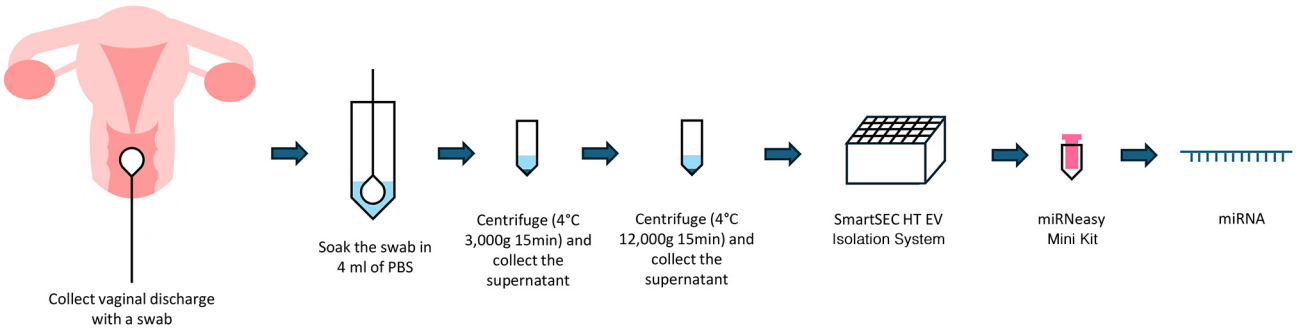

Figure 1. *Extraction of exosomal miRNA from vaginal discharge. Vaginal discharge was collected with a swab and soaked in 4 ml of PBS. The swabs were centrifuged at 3,000 × g for 15 min at 4˚C, and the supernatant was collected. The swab was then centrifuged again at 12,000 × g for 15 min at 4˚C, and that supernatant was collected. Exosomes were extracted using the SmartSEC HT EV Isolation System. miRNA was extracted using an miRNeasy Mini Kit.*

exosomes was analyzed using a RNA 6000 Pico Kit (Agilent Technologies). Most of the RNA contained in exosomes was small RNA less than 200 nt (Figure 2).

qRT-PCR. A TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was used to create cDNA from total RNA, according to the manufacturer's protocol. cDNA was stored at –20˚C until further use. qPCR was performed in triplicates using diluted cDNA with the TaqMan® Universal Master Mix II, no UNG (Applied Biosystems) and TaqMan® Fast Advanced Master Mix (Applied Biosystems). When comparing miRNA expression, syn-cel-miR-39, an external standard, was used to normalize the data according to previous reports (9, 17, 18). The ΔΔCt method was used to compare miRNA expression levels.

Statistical analysis. The Mann-Whitney *U*-test was used to examine the differences in miRNA expression levels (the ΔΔCt value) between the benign and malignant groups. The Spearman's correlation coefficient was used to examine any correlation between the ΔΔCt value and patient age or CA125 value. The Kruskal-Wallis test was used to examine whether there were significant differences in ΔΔCt values by histological type in the malignant group. The Mann-Whitney *U*-test was also used to examine whether there were significant differences in ΔΔCt values based on lymph node metastasis, BRCA, or HRD status in the malignant group. *p*-Values of 0.05 or less were considered significant. Statistical analysis was performed using EZR version 1.66 (Jichi Medical University Saitama Medical Center, Saitama, Japan).

Functional enrichment analysis. To investigate the target genes of miR-575, we used the miRNA database miRDB (https://mirdb.org/, accessed on January 28, 2025). To analyze which biological processes those target genes are involved in, Gene Ontology enrichment analysis was performed using FunRich (version 3.1.4).

Approval of the research protocol by an Institutional Reviewer Board. Ethical consent was granted from the Ethical Committee Review Board of the Clinical and Translational Research Center of Kobe University Hospital (permit number: No. B220071)

Informed consent. Because this study was an observational study using existing samples and information at the Kobe University, written consent was not sought from individual research subjects, but for those research subjects who had an opportunity to receive an explanation after the date on which the head of the research institution gave permission to conduct the study, an information disclosure document was provided, which was used to provide an easy-to-understand explanation, and oral consent was obtained and recorded in the patient's chart. Information regarding this study was also appropriately disclosed, and research

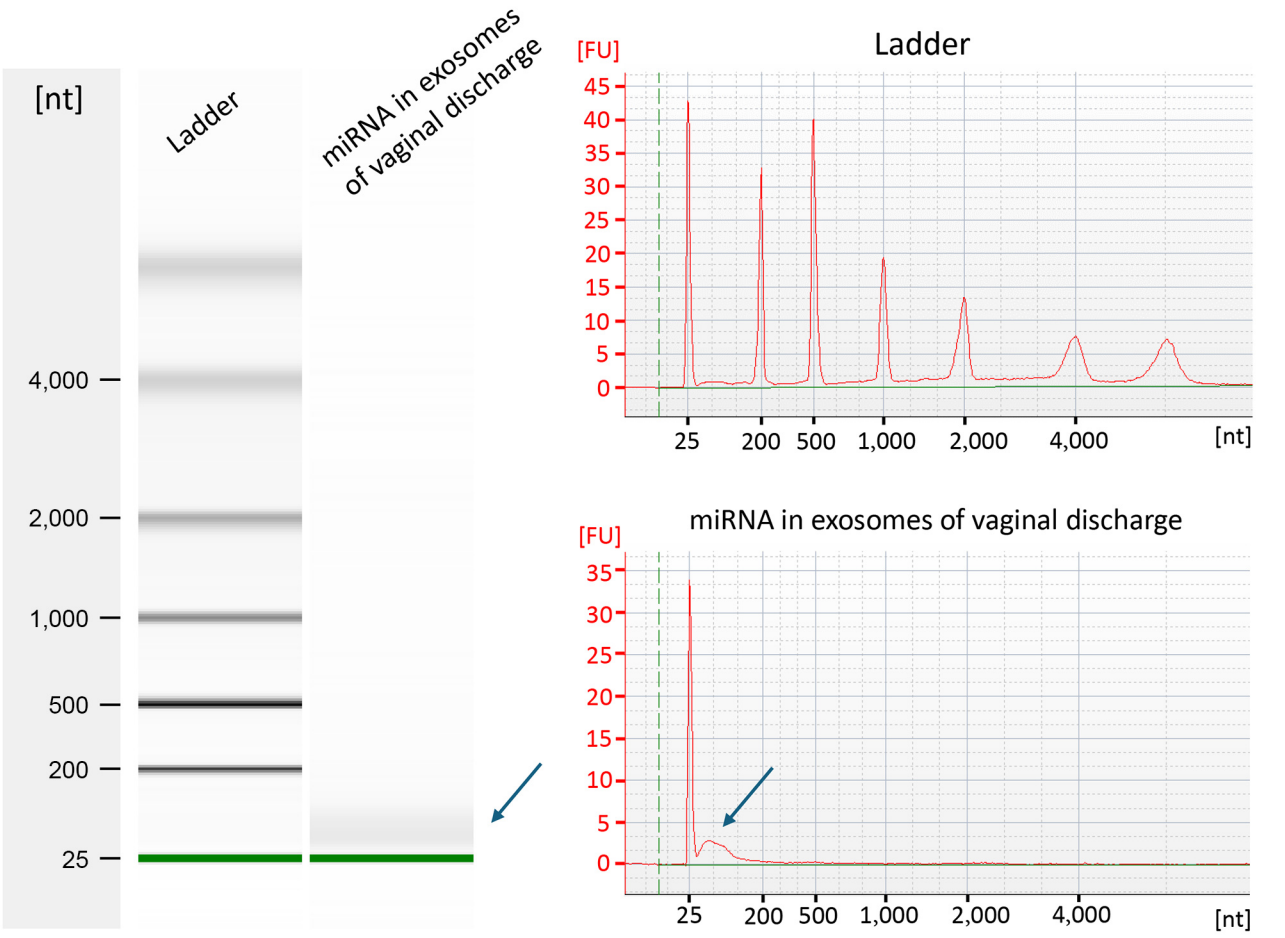

Figure 2. *MiRNA extracted from the exosomes of the vaginal discharge was analyzed using a Bioanalizer 2100 instrument. Most of the RNA contained in the exosomes was small RNA less than 200 nt. nt, Nucleotide.*

subjects were given the opportunity to refuse to register for this study. Opportunities for refusal in this study were handled by phone or email.

Results

Microarray analysis of miRNAs. Of the 2632 miRNAs, 538 were considered to have significant differences. Of the 538 miRNAs, 144 were selected that had a global normalization of 100 or higher in at least one of the four samples to exclude miRNAs with weak or almost no signal intensity. Additionally, of the 144 miRNAs, 25 that have been previously reported to be related to ovarian cancer were selected. Of the 25 miRNAs, 14 were selected for which papers reported data on qPCR in human samples (Figure 3 and Figure 4).

Comparison of relative miRNAs expression of vaginal discharge samples between malignant and benign groups. RT-qPCR was performed for four miRNAs: miR-575, miR-572, miR-1290 and miR-21. MiR-575, miR-572 and miR-1290 were the three miRNAs that the probe worked on out of 14 miRNAs. MiR-21 was not significantly different according to the miRNA microarray analysis but was additionally performed because it has previously been reported as a possible cancer-related miRNA. In the miRNA microarray, all four miRNAs were upregulated in the malignant group.

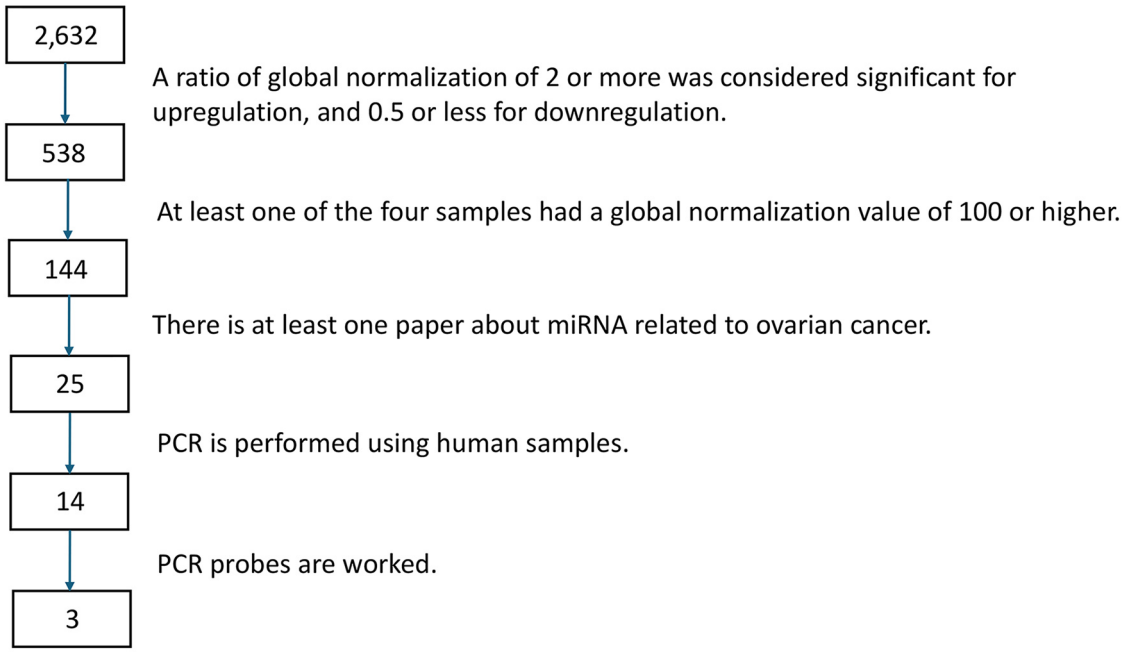

Figure 3. *Of the 2,632 miRNAs examined by miRNA microarray, 538 were considered to have significant differences. Furthermore, 144 were selected for which the global normalization value was 100 or higher in at least one of the four samples submitted to the array. Of these, 25 miRNAs were selected for which there was at least one ovarian cancer-related paper published. 14 miRNAs were selected for which PCR was performed on human samples. Three miRNAs were determined for which the PCR probe worked.*

RT-qPCR was finally performed on 30 malignant and 29 benign samples, the difference due to one sample being used up in the benign group. MiR-575 was strongly downregulated with significant differences in the malignant group, whereas the expression of three miRNAs (miR-572, miR-1290, and miR-21) did not show significant differences between the malignant and benign groups (Figure 5).

Relative miR-575 expression and patient background of malignant group. The qPCR results were analyzed with respect to age, CA125, histological type, stage, presence or absence of lymph node metastasis, and BRCA and HRD status; however, no significant differences were found (Figure 6 and Figure 7).

Target genes and functional enrichment analysis. A search for miR-575 in miRDB identified 118 miRNAs with a Target Score of 70 or higher, of which 116 were available in the Gene Ontology database, and 109 were available in the Biological process database (Supplementary Table I). The corrected *p*-value for "positive regulation of epithelial cell migration" was less than 0.05 for both the Bonferroni and Benjamini-Hochberg (BH) methods (p=0.046, 0.036). The only items that showed significant differences only with the BH method were "CD8-positive, alpha-beta T cell lineage commitment" (p=0.036) and "regulation of sarcomere tissue" (p=0.036) (Figure 8, Figure 9 and Table II).

Discussion

There is one report on miR-575 in ovarian cancer where miRNA expression was profiled using miRNA microarrays, and it was reported to be one of the miRNAs significantly upregulated in tumor tissues of recurrent ovarian high grade serous carcinoma (HGSC) (19). MiR-575 has been reported in other carcinomas, and in some cases it has been upregulated in cancer tissues and cell lines, while in others it has been down-regulated. Qin *et al.* found that inhibiting

	global normalization				Ratio
	E9	N3	OVK11	OVK13	
hsa-miR-575	680	45	1515	1131	5.62
hsa-miR-572	19	19	378	22	3.39
hsa-miR-6780b-5p	2607	560	8415	2569	2.88
hsa-miR-762	3361	1215	13057	4406	2.81
hsa-miR-638	3272	1418	14434	4475	2.79
hsa-miR-1290	185	9	251	75	2.48
hsa-miR-1246	696	29	776	262	2.37
hsa-miR-149-3p	1152	883	3009	2750	2.14
hsa-miR-4454	1093	2834	2392	342	0.38
hsa-miR-7977	388	1198	744	104	0.31
hsa-miR-4443	271	260	85	136	0.30
hsa-miR-1260a	121	359	115	49	0.27
hsa-miR-223-3p	155	27	19	11	0.16
hsa-miR-126-3p	175	-	-	-	0.06

Figure 4. *Global normalization and ratio of 14 miRNAs selected by microarray analysis. A ratio of 2 or more indicates up-regulation in malignant samples compared to benign samples, and a ratio of less than 0.5 indicates down-regulation.*

miR-575 reduced the proliferation and invasion of gallbladder cancer cells. They also found that p27Kip1 was a direct target of miR-575 by luciferase reporter assay (20). Liu *et al.* found that patients with high miR-575 expression in breast cancer had significantly poorer outcomes than those with low miR-575 expression. Tamoxifen treatment also downregulated miR-575 expression in ER-positive breast cancer. Overexpression of miR-575 reduced tamoxifen sensitivity by targeting CDKN1B and BRCA1 (21). Wang *et al.* reported that miR-575 was upregulated in gastric cancer tissues and cell lines, and PTEN was found to be a downstream target of miR-575 by luciferase reporter assay (22). Zhang *et al.* reported that RPL34-AS1 acts as a competitive endogenous RNA (ceRNA) of miR-575 in esophageal squamous cell carcinoma, alleviating the inhibitory effect of miR-575 on the target gene ACAA2, and suppresses the tumorigenesis of esophageal squamous cell carcinoma (23). These four reports indicate that miR-575 is upregulated in cancer and poor prognosis cases, but there are also reports showing the opposite. Wang *et al.* found that RIPK4 was increased in colon cancer tissues and cell lines, and RIPK4 was negatively regulated as a downstream target of miR-575 by luciferase reporter assay. MiR-575/RIPK4 axis inactivates the Wnt/β-catenin pathway through the downregulation of RUNX1, thus suppressing the progression of colorectal cancer (24). Huang *et al.* found that miR-575 suppresses tumor growth and metastasis by negatively regulating its downstream target DRP1 in head and neck cancer (25). Previous reports have also shown that miR-575 is upregulated in cancer tissues and cell lines, but there have been other reports of it being down-regulated, as in our study.

There have been two reports on exosomal miR-575, but neither were reports on malignant tumors. One reported that exosomal miR-575 was significantly downregulated in the breast milk from obese mothers, compared to normal weight mothers (26). The other reported that serum exosomal miR-575 was significantly

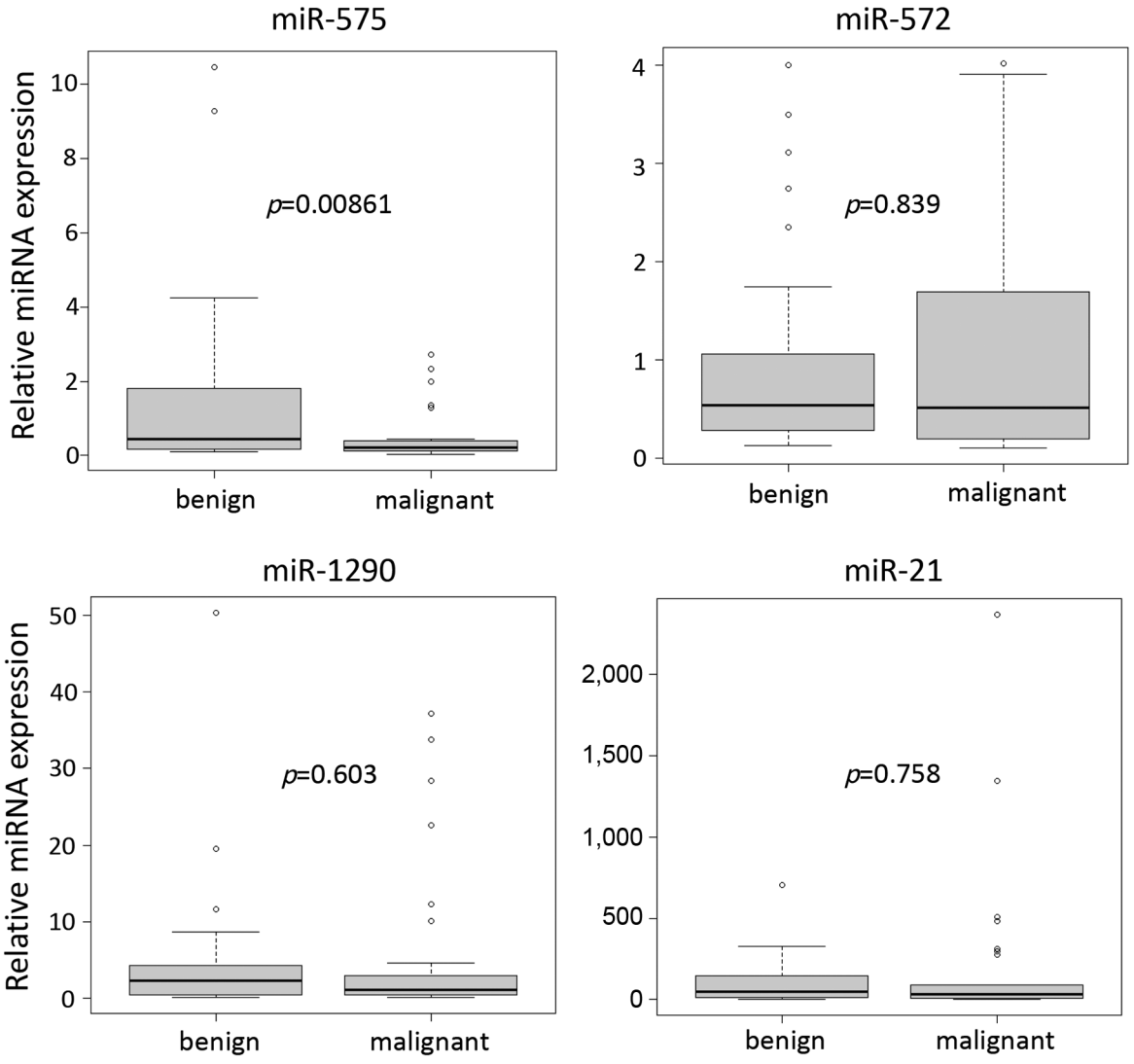

Figure 5. *Comparison of the relative levels of miRNAs in patients with benign gynecological disease and ovarian cancer. The expression of miR-575, miR-572, miR-1290, and miR-21 in exosomes from vaginal discharge was examined by qRT-PCR and assessed using the ΔΔCt method. Data were statistically analyzed by the Mann-Whitney U test and presented as box plots. The horizontal line represents the median. The height of the box is the interquartile range, which represents the 75th to 25th percentile of individual changes.*

downregulated in patients with Kawasaki disease and upregulated after IVIg (27). As mentioned above, there have been reports of miR-575 directly extracted from tissues, cell lines, and serum in several carcinomas, but exosomal miR-575 has never been reported in the context of malignancies such as ovarian cancer. This is the first time such information has been reported (20-25, 28, 29).

MiR-575 was upregulated in the microarray but downregulated in the RT-qPCR test results. When we checked with the analysis contractor, we were told that there are cases where the results of the array and PCR do not match and that the reasons are several. One is the difference in the correction method. The data of the array is corrected (standardized) using the expression level of the whole gene

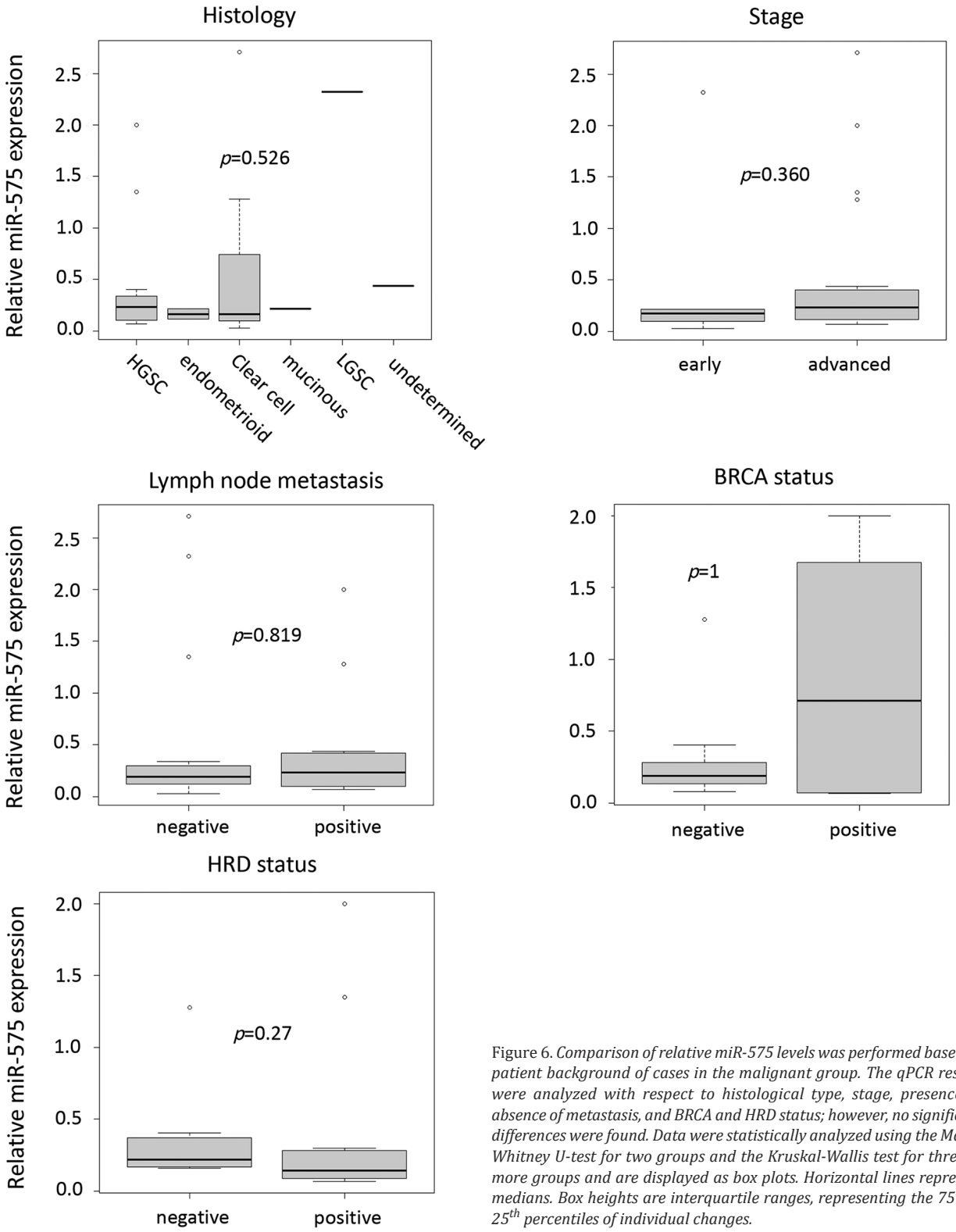

Figure 6. *Comparison of relative miR-575 levels was performed based on patient background of cases in the malignant group. The qPCR results were analyzed with respect to histological type, stage, presence or absence of metastasis, and BRCA and HRD status; however, no significant differences were found. Data were statistically analyzed using the Mann-Whitney U-test for two groups and the Kruskal-Wallis test for three or more groups and are displayed as box plots. Horizontal lines represent medians. Box heights are interquartile ranges, representing the 75th to 25th percentiles of individual changes.*

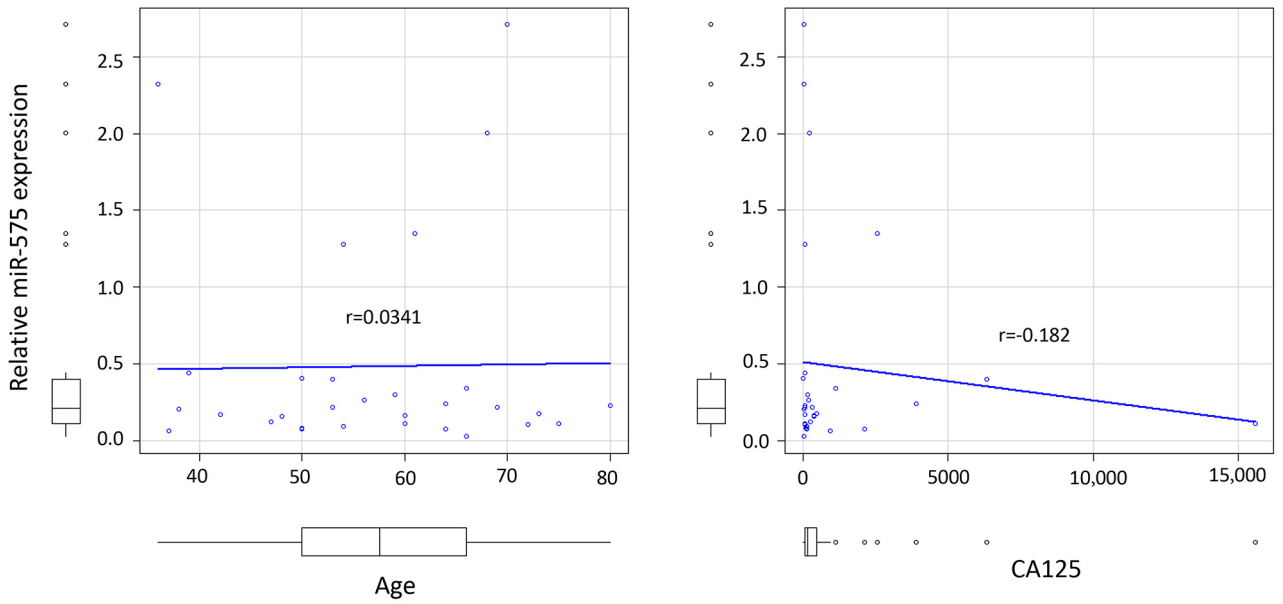

Figure 7. *Statistical examination of correlation between relative expression of miR-575 and age or CA125 levels using the Spearman's rank correlation coefficient. No clear correlation was found. Data were statistically analyzed also using the Spearman's rank correlation coefficient and are presented as box plots and regression lines. Horizontal lines in box plots represent medians. Box heights are interquartile ranges, representing the 75th to 25th percentiles of individual variation.*

by a method called global normalization; however, in the case of PCR, correction is usually performed using a specific gene or spike. Therefore, the difference in the correction method between the array and PCR may be the cause of the difference in the results (30, 31). The other is the influence of the difference in the detection method. For miRNA in body fluids, there are miRNAs called isomiRs, which are sequences with a few base sequences that are slightly different at the end of the miRNA. For such isomiRs, the array detects them by allowing subtle differences in the base sequence at the end; however, PCR does not allow subtle differences in the base sequence at the end and cannot detect them. As several papers have reported, isomiRs are more abundant than canonical miRNA subtypes (32, 33). Such differences in the detection method may affect the difference in the results of the array and PCR (34).

The advantage of diagnosing ovarian cancer from vaginal discharge is that it allows for liquid biopsy relatively close to the affected area. Shao *et al.* investigated the long non-coding RNA (lncRNA) AA174084 in gastric juice and reported that, although there was no difference in the concentration of AA174084 in plasma between gastric cancer patients and control groups, it could be detected at significantly higher concentrations in gastric juice (35). The vagina and peritoneal cavity are also connected *via* the uterus and fallopian tubes, so it may be possible to detect substances secreted from primary tumor tissue at higher concentrations.

A search in the miRDB database revealed that PTEN had a target score of 78% for miR-575, and the Gene Ontology analysis by FunRich also showed that PTEN was listed as a mapped gene name for two of the top 10 related biological processes. It is also interesting to note that MDM4, which has a p53-binding domain at its N-terminus, also had a Target score of 78% in miRDB, although it was not listed in the mapped gene names of biological process. RIPK4 had a target score of 75% and was also listed as a mapped gene name for the chromatin remodeling process among the top 10 related biological processes in FunRich's Gene Ontology analysis.

Table II. *Gene Ontology analysis of miRDB for miR-575 was performed using FunRich.*

Biological process	Corrected *p*-values						
	Percentage of genes	Fold enrichment	*p*-Value (Hypergeo-metric test)	Bonferroni method	BH method	Q-value (Storey-Tibshirani method)	Mapped gene names
Positive regulation of epithelial cell migration	3.66972477	38.21880503	3.71836E-06	0.045691186	0.036445173	0.233124323	DOCK5; ITGA2; EPB41L5; CAPN7
Regulation of sarcomere organization	1.83486239	333.6759013	8.89775E-06	0.109335519	0.036445173	0.233124323	BCL2; TOX
CD8-positive, alpha-beta T cell lineage commitment	1.83486239	333.6759013	8.89775E-06	0.109335519	0.036445173	0.233124323	ANKRD23; AKAP13
Positive regulation of sodium ion transport	2.75229358	59.04552986	1.72628E-05	0.212125572	0.053031393	0.339219342	ANK3; WNK3; CNTN1
Positive regulation of sodium ion transmembrane transporter activity	1.83486239	167.2540054	5.31773E-05	0.65344276	0.130688552	0.835959271	ANK3; WNK3
Chromatin remodeling	10.0917431	4.068362115	8.7353E-05	1	0.155276618	0.993238705	WEE1; CSNK1G3; RIPK4; ZNF827; USP49; CHD9; WNK3; TSSK1B; PTEN; PHF8; EPHB1
Ventricular cardiac muscle cell differentiation	1.83486239	133.8699724	8.84551E-05	1	0.155276618	0.993238705	MEF2C; NRG1
Central nervous system neuron axonogenesis	1.83486239	111.5954345	0.000132423	1	0.203401134	1	NDEL1; PTEN
Positive regulation of cation channel activity	1.83486239	95.6759717	0.000185028	1	0.22736289	1	COMT; EDN1
GO:1902074	1.83486239	95.6759717	0.000185028	1	0.22736289	1	ANK3; EDN1

The top 10 biological processes were summarized with percentage of genes, *p*-values, and mapped gene names. BH, Benjamini-Hochberg.

In this study, miR-575 was downregulated in malignant samples. However, further research is needed to determine the mechanism by which it is downregulated and its function in ovarian cancer. Since it was suggested that miR-575 may be significantly involved in the positive regulation of epithelial cell migration, we plan to continue research into migration and invasive abilities using cell lines. The results of this study are very interesting and promising, but their usefulness is limited by the small number of samples used in this study. In the future, we

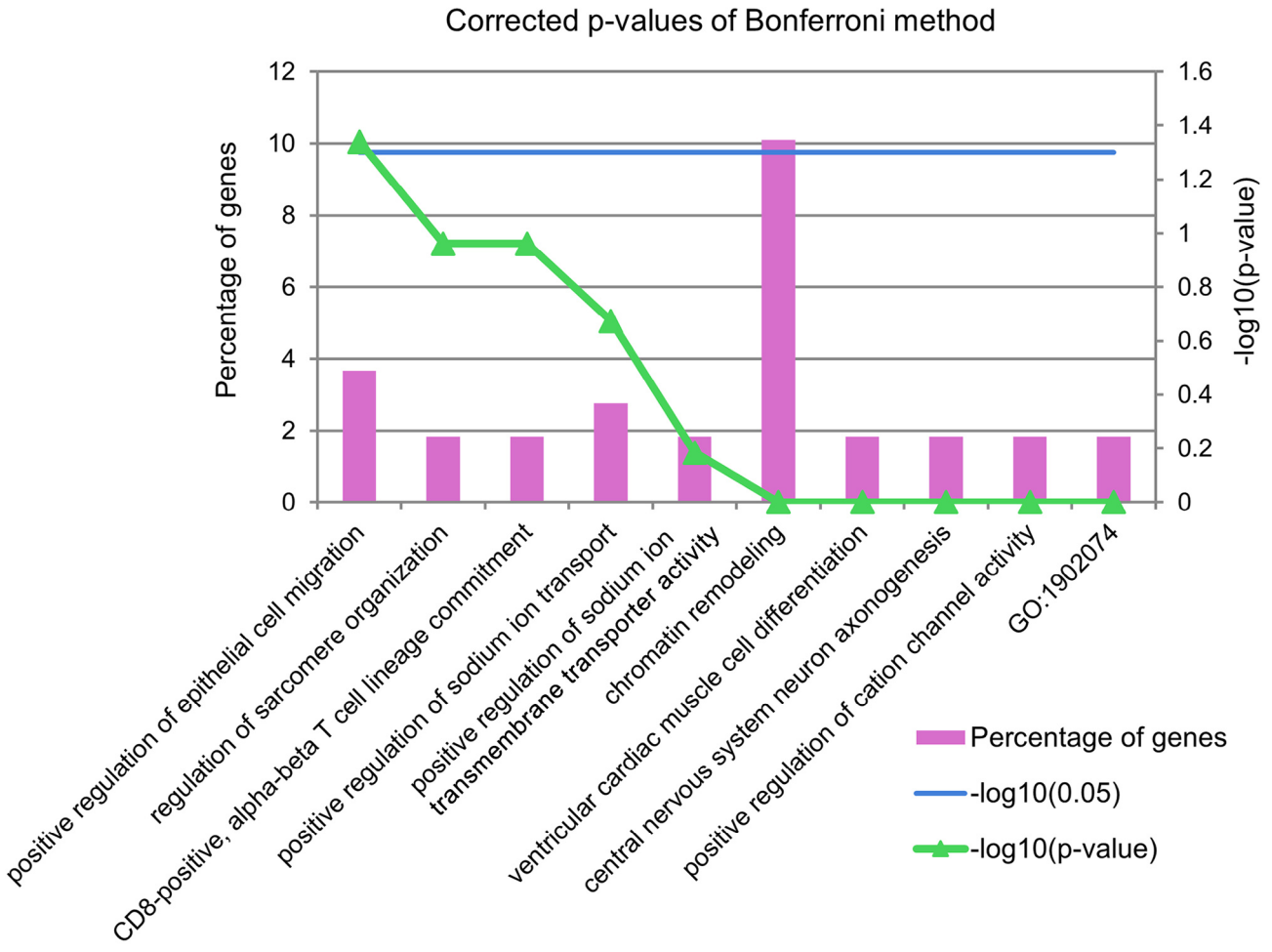

Figure 8. *Corrected p-values of Bonferroni method. The primary axis represents the percentage of genes, and the secondary axis is -log10(p-value). The blue line indicates the p=0.05 line. The bar graph shows the percentage of genes for each biological process.*

plan to analyze more vaginal discharge exosomes by dividing the histological type and stage of ovarian cancer and analyze whether they contribute to prognosis and diagnosis. If further research can be used for early detection, samples could be collected at the same time as cervical cancer screening, which could reduce the burden on patients.

Conclusion

This is the first study to investigate exosomal miRNAs in vaginal discharge of ovarian cancer. Exosomal miR-575 in vaginal discharge may be used as a biomarker for ovarian cancer.

Supplementary Material

Available at: https://figshare.com/articles/online_resource/Gene_Ontology_Analysis_of_miR-575_target_genes_xlsx/28360088?file=52178723

Conflicts of Interest

The Authors declare no potential conflicts of interest in relation to this study.

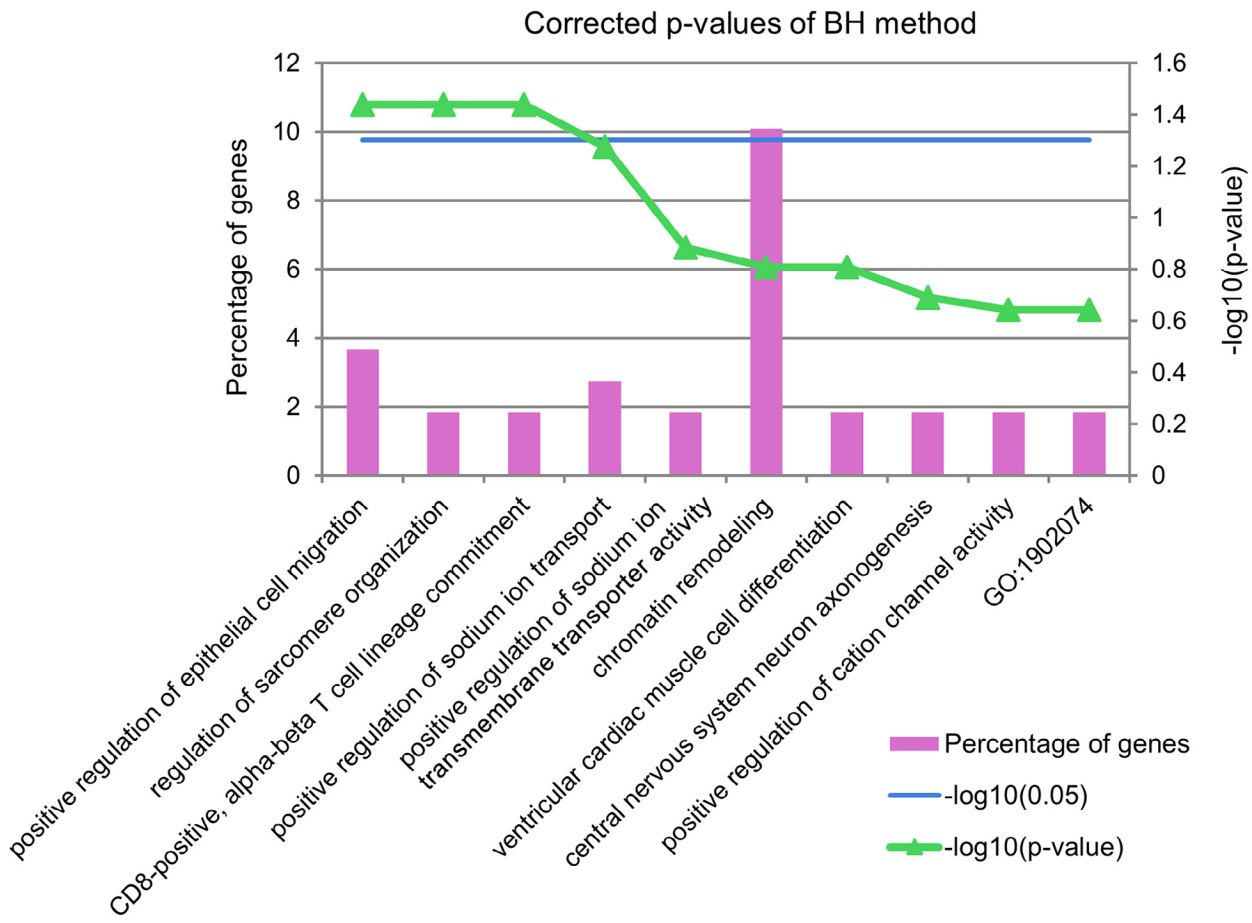

Figure 9. *Corrected p-values of BH method. The primary axis represents the percentage of genes, and the secondary axis is -log10(p-value). The blue line indicates the p=0.05 line. The bar graph shows the percentage of genes for each biological process. BH, Benjamini-Hochberg.*

Authors' Contributions

MA: sample collection; data curation; investigation; methodology; writing – original draft. SI: research technology guidance; supervision; writing – review and editing. YY: data curation. KO: sample collection. KY: sample collection. YT: Supervision. All Authors read and approved the final manuscript.

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