RESEARCH ARTICLE

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MIR-379-5p Expression in Endometrial Cancer and Its Correlation with ROR1 Expression

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Abstract

Objective: To asses miR-379-5p expression in endometrial cancer (EC) and its correlation with ROR1 expression and to investigate the relation between miR-379-5p and ROR1 expressions and the clinicopathological picture of EC. **Methods:** Fifty female of EC were joined to this study. The gene expression of miR-379-5p (by quantitative real time-PCR) and ROR1 (by quantitative real time-PCR and immunohistochemistry) were studied in EC and normal nearby endometrial tissue. **Results:** The gene expression of miR-379-5p was significantly downregulated while that of ROR1 was significantly upregulated in EC tissues compared to adjacent normal endometrial tissues. Furthermore, miR-379 and ROR1 expressions significantly associated with tumor stage ($P< 0.045$), grade ($P< 0.001$), myometrial invasion ($P <0.001$) and LN metastasis ($P< 0.034$). In addition, miR-3795p and ROR1 gene expression were negatively correlated ($r = -0.746$, $P < 0.001$). **Conclusions:** In EC, miR-379-5p can be used as a diagnostic marker, and ROR1 could be a potential target of miR-379-5p.

Keywords: Endometrial carcinoma, miR-379-5p, ROR1, Immunohistochemistry, Prognosis, PCR

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Introduction

Endometrial cancer (EC) is the most prevalent cancer of the female reproductive organs and the world's sixth most common cancer, in 2020, new cases about 417, 367 and 97,370 deaths due to EC worldwide as, the GLOBOCAN database (Ferlay et al., 2021). The rate of occurrence has risen dramatically in the previous decade, especially in developed countries with an incidence of 5.9% (Jansen et al., 2022). Genetic predisposition is increasingly acknowledged as a major component in endometrial cancer risk, in addition to reproductive variables and excess weight (Dork et al., 2020). Patients with metastatic and recurrent endometrial cancer have a dismal prognosis (Morice et al., 2016). As a result, more research into novel targets and new therapeutics, as well as more exploration of the molecular process of endometrial cancer, is required to prevent its formation and progression.

MicroRNAs (miRNAs) are single-stranded non-coding RNAs with a length of 19–25 nucleotides that are produced by a multistep process that starts in the nucleus and ends in the cytoplasm (Hammond, 2015; Di Leva et al., 2017). MiRNAs are partially complementary to one or more mRNA molecules (Gebert et al., 2019) and function as post-transcriptional modulators of target genes via complementary pairing with the target genes' sequence (Liolios et al., 2019). They work by repressing gene expression in a variety of mechanisms, including mRNA cleavage and de-adenylation, as well as translational repression (Liu and Li 2014). MiRNAs have been shown in numerous studies to have an important role in cancer progression and treatment. As a result, the expression pattern, roles, and associated mechanisms of miRNAs in EC should be explored to find new and effective EC therapeutics. MiR-379-5p is part of a big miRNA gene cluster on human chromosome 14q32 (Zhao and Chu 2018). Several studies have found that miR-379-5p suppresses tumour growth in a variety of cancers, including osteosarcoma, bladder cancer, and melanoma (Wu et al., 2017; Xie et al., 2017). MiR-379 regulates a variety of biological processes, including proliferation, cell cycle, apoptosis, and metastasis, and is downregulated in a variety of malignancies (Jiang et al., 2018; Xu et al., 2017). However, the expression profile of miR-379 in endometrial cancer has yet to be determined, as well as

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its role in this malignancy.

ROR1 is a tyrosine kinase-like orphan receptor with important functions during embryogenesis. Because ROR1 has been found to be overexpressed in a variety of malignancies compared to its low expression in healthy adult tissue, it is being evaluated as a potential target for cancer treatment (Chien et al., 2016).

ROR1 has been linked to cell proliferation, stemness (Liu et al., 2015), epithelial-mesenchymal transition (EMT) (Cui et al., 2012), and other metastatic properties in a variety of cancer types (Henry et al., 2015). It is thought to play a role in oncogenesis by triggering cell survival signaling events, notably the non-canonical WNT signalling pathway (Zhao et al., 2021). Furthermore, in an EC cell line, Liang et al. found a link between ROR1 expression and miR-379-5p. ROR1 could be a new miR-379-5p target, but they did not study the levels of expression in fresh tissue specimens or connect them with clinicopathological characteristics (Liang et al., 2021). As a result, the goal of this work was to investigate the expression of miR-379-5p and ROR1 in EC tissues, as well as the relationship between the two markers. We also wanted to see if there was a link between the levels of miR-379-5-p and ROR1 and the clinicopathological characteristics of EC patients.

Materials and Methods

Patients' selection

This study was conducted at Medical Biochemistry and Molecular Biology, Pathology, Clinical Oncology, Gynecology & Obstetrics between January 2017, and August 2021.

Patients were pathologically proven endometrial carcinoma and complete surgical staging, ECOG performance score ≤ 2, adequate renal, hepatic, and hematological functions were evaluated. A total of fifty patients of EC were joined this study after exclusion of cases with previous radiotherapy or chemotherapy before surgery. Informed consent was obtained from all participants in the study. The studied cases were diagnosed via CT and/or MRI. Full history taking and clinical examination to all cases were done. Fresh endometrial tissues (cancer and adjacent non-malignant tissues) were taken at surgery and divided into 2 parts. 1st part was frozen and maintained at −80◦C for RNA extraction. The 2nd part was preserved in formalin solution for histopathological and immunohistochemistry studies. The tumor grade and stage were assessed according to FIGO surgical staging system (Creasman, 2009).

Treatment schedules

The surgery was the primary treatment and was performed as total abdominal hysterectomy and bilateral salpingo-oophorectomy with lymphadenectomy and full surgical staging. Adjuvant treatment of EC depends on the risk of relapse. Well-established clinicopathological risk factors are age, FIGO-stage, depth of myometrial invasion, histological type, grade, and lymph-vascular space invasion (LVSI).

Patients with disease of grade 1 endometrioid histology and uterine-limited disease with <50% myometrial invasion (stage IA), were deemed to be low-risk and observation was preferred. On the other hand, if the tumor is grade 3, LVSI is present, or the patient is ≥ 60 years old, in this case vaginal cuff brachytherapy was the treatment of choice.

High-risk patients are those with stage III or higher, regardless of histology or grade. The primary treatment for patients with stage III-IV endometrioid endometrial carcinomas is systemic chemotherapy with six cycles of carboplatin and paclitaxel. Likewise, all patients with serous and clear cell histology were considered at high-risk, regardless of the stage and treated with systemic chemotherapy with or without radiotherapy.

An Intermediate risk includes all others, and they were treated with vaginal brachytherapy, and /or external beam radiotherapy. External beam RT was delivered with 3-dimensional conformal technique (3DCRT) utilizing 50.4 Gy to the pelvis over five weeks with a daily fraction size of 1.8 Gy. EBRT was directed via a 4-field technique using a 10-MV linear accelerator with multi-leaf collimator with conventional fractionation delivers in 25–28 days. The treatment fields either involved the pelvic lymphatics or pelvic and paraaortic (PA) lymphatics starting from T12–L1 interspace cranially. In patients with paraaortic LN metastasis, PA fields were irradiated.

Treatment planning was achieved by the CT-based three-dimensional conformal radiotherapy modality. The clinical target volume (CTV) entails of the upper area of the vaginal stump and regional lymphatic drainage regions, including common iliac, internal iliac, external iliac, obturator, and presacral areas.

Follow-up

The follow-up regimen included clinical/pelvic examination every three months for 2 years then every six months. At each follow-up, symptoms were documented, and abdominal palpation and pelvic examination were completed. Vault smears were done every six months / the first 2 years, then annually. Pelvic and abdominal ultrasound or CT and chest X-ray or CT were taken. Treatment toxicities were evaluated according to "Common Terminology Criteria for Adverse Events" version 4.0. Radiation related gastrointestinal, genitourinary, dermatologic, and hematologic toxicities were evaluated.

RNA extraction and real-time PCR

Total RNA with miRNAs from tumor and normal endometrial tissues was extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany). By using the Power cDNA synthesis Kit (iNtRON Biotechnology, Seongnam, Korea), reverse transcription of RNA was done and quantitative Realtime PCR was performed using Mx3005PTM (Stratagene, La Jolla, CA, USA) following the manufacturer's protocol. The following thermocycling conditions were used for the qPCR: initial denaturation and polymerase activation for 15 min at 95 ◦C, then 40 cycles of denaturation for 15 sec at 94◦C; annealing for 30 sec at 55◦C; and lastly; extension for 30 sec at 70◦C. Twenty uL volume reactions were done with

5 uL of cDNA, 100 pmol/uL of each primer (0.5 uL each) (Biolegio, Netherlands), 10 uL of PCR EvaGreen Master Mix (Jena Bioscience GmbH, Jena, Germany) and 4 uL distilled water. MiR- 379 expression was normalized to U6 while ROR1 expression was normalized to a β-actin s housekeeping gene. The cycle threshold (Ct) values were calculated. The level of the studied genes was normalized by calculating the ΔCt value. The amplitude of change of the expression (fold change) in cancerous tissues relative to the control tissues was analyzed by the $2^{-\Delta\Delta C}$t equation. The relative expression was analyzed by the $^{2-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The primers used in the present study were as follows:

miRNA 379 (forward, 5'-GCGCTTATTGCTTAAGAATAC 3', and reverse, 5' CAG TGCAGGGTCCGAGGT-3') and U6 (forward, 5'-GCTTCG GCAGCACATATACTAAAAT-3', and reverse, 5'CGCTTCACGAATTTGCGTGTCAT-3)' ROR1 (forward), 5′-AGATCACAGCTGCCTTCACTAT-3′; ROR1 (reverse), 5′-GACATTCTCCAGGATTTCACAT-3′; β-actin (forward), 5′-GGCGGCACCACCATGTACCCT-3′; β-actin (reverse), 5′-AGGGGCCGGACTCGTCATACT-3′.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks were cut into 3–5 μm sections followed by deparaffinization and rehydration. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0) at the microwave for 20 min. Hydrogen peroxide 3% for about 10 min to block the endogenous peroxidase activity was done. After repeated washing in PBS, the slides were incubated with rabbit anti-ROR1 monoclonal antibody (1:50, #564464, BD Biosciences, USA). The binding site of primary antibodies was detected using the polymer detection system; the Dako EnVision™ kit (Dako, Copenhagen, Denmark). Lastly, the sections were counterstained, dehydrated, and then mounted.

Assessment of ROR1 immunohistochemical expression: ROR1 immunostaining was graded as follows: 0 (Negative), 1 (Weak), 2 (Moderate) and 3 (Intense).

Statistical analysis of the data

Our data was analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). Kolmogorov-Smirnov verified the normality of variables distribution, Student t-test compared two categories for normally distributed quantitative variables, while ANOVA was used for comparing the studied categories. Pearson coefficient correlated between two normally distributed quantitative variables. Kaplan-Meier Survival curve was used for the significant relationship with progression free survival (PFS) and overall survival (OS). Significance of the obtained results was judged at the 5% level.

Results

Patient characteristics

This study included 50 females with EC, with mean age ± SD (54.70 ± 5.95). The age ranged from 44 to 66

Table 1. Distribution of the Studied Cases According to Age mean+-SD, Grade No. (%), Histological type No. (%), Stage No. (%), Myometrial invasion No. (%), LVSI, L.N metastasis No. (%), Adjuvant treatment No. (%), RT No. (%), Chemotherapy No. (%), ROR1 (IHC) No. (%), ROR1 (IHC) No. (%) and RORI.

Variable	Mean ± SD. Median (Min. – Max.)	No. (%) (n = 50)
Age (years)		
Mean ± SD.	54.70 ± 5.95	
Median (Min. – Max.)	54 (44 – 66)	
Grade No. (%)		
G1		12 (24.0%)
G2		18 (36.0%)
G3		20 (40.0%)
Histological type No. (%)		
Serous carcinoma		6 (12.0%)
Clear cell carcinoma		3 (6.0%)
Endometrioid		41 (82.0%)
Stage No. (%)		
Stage I		22 (44.0%)
Stage II		17 (34.0%)
Stage III		7 (14.0%)
Stage IV		4 (8.0%)
Myometrial invasion No. (%)		
< 50%		19 (38.0%)
> 50%		31 (62.0%)
LVSI		
Absent		20 (40.0%)
Present		30 (60.0%)
L.N metastasis No. (%)		
Absent		39 (78.0%)
Present		11 (22.0%)
Adjuvant treatment No. (%)		
Absent		9 (18.0%)
Present		41 (82.0%)
RT No. (%)		
No		2 (4.0%)
Observation		9 (18.0%)
EBRT		18 (36.0%)
Vaginal brachytherapy		8 (16.0%)
EBRT + Vaginal brachytherapy		13 (26.0%)
Chemotherapy No. (%)		
Absent		33 (66.0%)
Present		17 (34.0%)
ROR1 (IHC) No. (%)		
Negative		10 (20.0%)
Weak		11 (22.0%)
Moderate		15 (30.0%)
Intense		14 (28.0%)
RORI		
Mean ± SD.	3.62 ± 1.56	
Median (Min. – Max.)	3.50 (1.50 – 7.10)	

Table 1. Continued

	Mean ± SD. Median (Min. – Max.)	No. (%)
miRNA 397-5p		
Mean ± SD.	0.39 ± 0.18	
Median (Min. – Max.)	0.39 (0.11 – 0.80)	
Failure No. (%)		
No Failure		40 (80.0%)
Failure		10 (20.0%)
Treatment failure		(n = 46)
None		36 (78.3%)
Mets / Lung only		2 (4.3%)
Mets / Multiple sites		4 (8.7%)
Mets / Peritoneal seeding		1 (2.2%)
Pelvic cavity only		1 (2.2%)
Pelvic / Para aortic LN's		2 (4.3%)
PFS (mo)	24.16 ± 9.03	
Mean ± SD.	24.0 (10.0 – 36.0)	
Median (Min. – Max.)		
OS status No. (%)		
Survival		44 (88.0%)
Death		6 (12.0%)
OS (mo)		
Mean ± SD.	27.24 ± 6.85	
Median (Min. – Max.)	28 (15 – 36)	

years. Grade 3 was the predominant grade (40.0%) and 82% was endometrioid cancer type. According to stage, the majority was stage I-II (78%). LVSI was present in 30 (60.0%) of the studied cases. L.N metastasis was present in 22 % of the studied cases. Adjuvant treatment was administrated to in 82.0% of the studied cases. There were 10 (20.0%) had Negative ROR1, 11 (22.0%) had Weak ROR1, 15 (30.0%) had Moderate ROR1 and 14 (28.0%) had Intense ROR1 (Figure 1). The mean RORI was 3.62 ± 1.56 SD with range (1.50 – 7.10). The mean of miR 379-5p was 0.39 ± 0.18 SD with range (0.11- 0.80). The different clinicopathological features of the studied cases were presented in Table 1.

Table 2. Correlation between Different Parameters (n = 50)

	r	p
RORI vs. miRNA 397-5p	-0.746	<0.001*
miRNA 397-5p vs. Age (years)	-0.458*	0.001*
RORI vs. Age (years)	0.308*	0.029*

r, Pearson coefficient; *, Statistically significant at $p \leq 0.05$

Association between miRNA 379-5p and RORI (PCR) with different parameters Table 2, 3

There was highly statistically significant difference with negative correlation between miRNA 379-5p and RORI (Figure 2). There was statistically significant difference with positive correlation between Age and RORI. There was statistically significant difference with negative correlation between Age and miRNA 379-5p.

There was highly statistically significant difference between both miRNA 3795p and ROR1 with grade, stage, myometrial invasion, LVSI, L.N metastasis, adjuvant treatment, RT, chemotherapy, treatment failure, and OS status. There was statistically insignificant difference between miRNA 379-5p and histological type of EC.

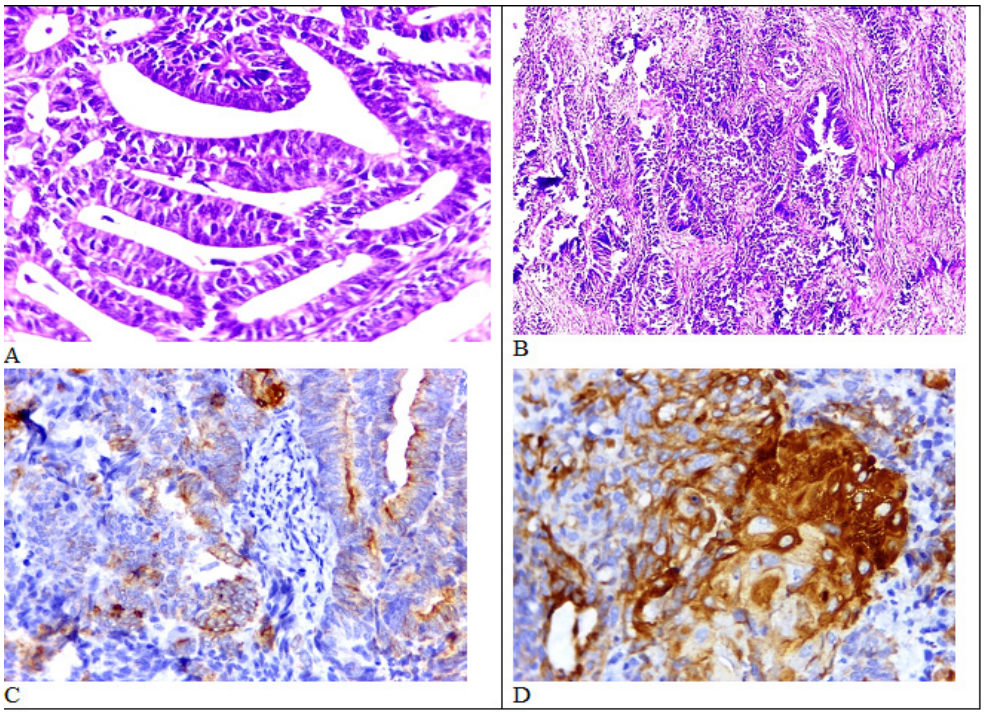

Figure 1. (A) A case of endometrioid carcinoma (EEC) GI (H& E× 400 magnification), (B) EEC GII showing myometrial invasion (IHC ×400 magnification), (C) EEC GI showing weak ROR1 immunoexpression (IHC ×400 magnification), (D) EEC GIII showing intense ROR1 immunoexpression (IHC ×400 magnification).

Table 3. Relation between miRNA and RORI with Different Parameters (n = 50)

	N	miRNA			RORI		
		Mean ± SD.	Median (Min. – Max.)	(p)	Mean ± SD.	Median (Min. – Max.)	(p)
Grade							
G1	12	0.57 ± 0.14	0.57 (0.32 – 0.80)		2.36 ± 0.81	2.15 (1.50 – 4.30)	
G2	18	0.43 ± 0.14	0.41 (0.19 – 0.77)	(p<0.001*)	3.65 ± 1.69	2.80 (1.90 – 7.10)	(0.001*)
G3	20	0.26 ± 0.12	0.25 (0.11 – 0.58)		4.35 ± 1.35	4.10 (1.60 – 6.50)	
Histological type							
Clear cell carcinoma	3	0.32 ± 0.23	0.26 (0.13 – 0.58)		3.83 ± 2.11	4.10 (1.60 – 5.80)	
Serous carcinoma	6	0.21 ± 0.05	0.20 (0.16 – 0.28)	(p=0.019*)	4.42 ± 1.08	4.05 (3.60 – 6.50)	-0.391
Endometrioid	41	0.42 ± 0.18	0.41 (0.11 – 0.80)		3.49 ± 1.58	2.90 (1.50 – 7.10)	
Stage							
Stage I	22	0.51 ± 0.15	0.47 (0.31 – 0.80)	(p<0.001*)	2.99 ± 1.35	2.55 (1.50 – 5.80)	
Stage II	17	0.35 ± 0.15	0.33 (0.12 – 0.65)		3.89 ± 1.70	3.80 (1.60 – 7.10)	(0.045*)
Stage III	7	0.24 ± 0.11	0.23 (0.11 – 0.45)		4.26 ± 1.23	4.10 (2.20 – 5.90)	
Stage IV	4	0.18 ± 0.08	0.16 (0.11 – 0.29)		4.80 ± 1.60	4.75 (3.20 – 6.50)	
Myometrial invasion							
< 50%	19	0.53 ± 0.15	0.53 (0.32 – 0.80)	t=5.241*	2.69 ± 1.16	2.40 (1.50 – 5.80)	
> 50%	31	0.31 ± 0.15	0.29 (0.11 – 0.65)	(p<0.001*)	4.18 ± 1.52	3.90 (1.60 – 7.10)	(0.001*)
LVSI							
Absent	20	0.52 ± 0.16	0.51 (0.28 – 0.80)	t=4.920*	2.68 ± 0.99	2.45 (1.50 – 4.90)	
Present	30	0.31 ± 0.15	0.29 (0.11 – 0.65)	(p<0.001*)	4.25 ± 1.57	4.10 (1.60 – 7.10)	(0.001*)
L.N metastasis							
Absent	39	0.44 ± 0.17	0.42 (0.12 – 0.80)	t=4.155*	3.38 ± 1.56	2.70 (1.50 – 7.10)	
Present	11	0.22 ± 0.10	0.19 (0.11 – 0.45)	(p<0.001*)	4.45 ± 1.32	4.10 (2.20 – 6.50)	(0.034*)
Treatment failure							
No Failure	40	0.43 ± 0.18	0.42 (0.11 – 0.80)	t=3.242*	3.33 ± 1.49	2.70 (1.50 – 6.50)	
Failure	10	0.24 ± 0.10	0.24 (0.11 – 0.42)	(p=0.002*)	4.77 ± 1.35	4.25 (2.90 – 7.10)	(0.005*)
OS status							
Survival	44	0.42 ± 0.17	0.41 (0.11 – 0.80)	t=6.700*	3.39 ± 1.45	2.90 (1.50 – 7.10)	
Death	6	0.18 ± 0.06	0.17 (0.11 – 0.29)	(p<0.001*)	5.27 ± 1.45	5.85 (3.20 – 6.50)	(0.008*)

Relation between RORI immunohistochemical expression and different parameters

There was highly statistically significant difference in ROR1 immunohistochemical expression regarding EC grade, myometrial. invasion, LVSI, L.N metastasis, treatment failure and OS status. There was insignificant

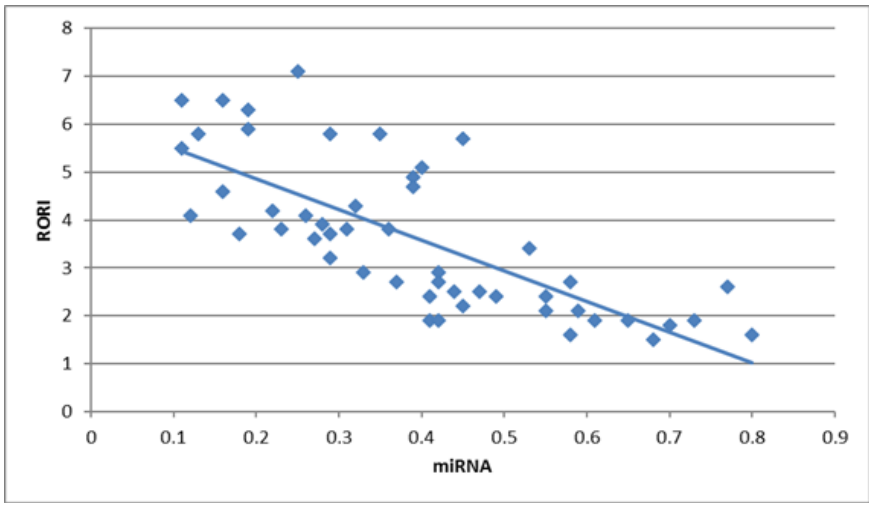

Figure 2. Correlation between RORI (PCR) and miRNA 379-5p

Table 4. Relation between RORI Immunohistochemical Expression and Different Parameters (n = 50)

	RORI				MC_p
	Negative (n = 10)	Weak (n = 11)	Moderate (n = 15)	Intense (n = 14)	
Grade					
I	8 (80%)	2 (18.2%)	2 (13.3%)	0 (0%)	<0.001*
II	1 (10%)	8 (72.7%)	5 (33.3%)	4 (28.6%)	
III	1 (10%)	1 (9.1%)	8 (53.3%)	10 (71.4%)	
Histological type					
Clear cell carcinoma	1 (10%)	0 (0%)	1 (6.7%)	1 (7.1%)	0.225
Serous carcinoma	0 (0%)	0 (0%)	2 (13.3%)	4 (28.6%)	
Endometrioid	9 (90%)	11 (100%)	12 (75%)	9 (64.3%)	
Stage					
Stage I	8 (80%)	6 (54.5%)	6 (40%)	2 (14.3%)	0.116
Stage II	2 (20%)	4 (36.4%)	5 (33.3%)	6 (42.9%)	
Stage III	0 (0%)	1 (9.1%)	3 (20%)	3 (21.4%)	
Stage IV	0 (0%)	0 (0%)	1 (6.7%)	3 (21.4%)	
Myometrial invasion					
< 50%	8 (80%)	6 (54.5%)	3 (20%)	2 (14.3%)	0.003*
> 50%	2 (20%)	5 (45.5%)	12 (80%)	12 (85.7%)	
LVSI					
Absent	8 (80%)	6 (54.5%)	5 (33.3%)	1 (7.1%)	0.002*
Present	2 (20%)	5 (45.5%)	10 (66.7%)	13 (92.9%)	
L.N metastasis					
Absent	10 (100%)	10 (90.9%)	11 (73.3%)	8 (57.1%)	0.057
Present	0 (0%)	1 (9.1%)	4 (26.7%)	6 (42.9%)	
Treatment failure					
No Failure	10 (100%)	11 (100%)	11 (73.3%)	8 (57.1%)	0.013*
Failure	0 (0%)	0 (0%)	4 (26.7%)	6 (42.9%)	
Mortality					
Survival	10 (100%)	11 (100%)	14 (93.3%)	9 (64.3%)	0.016*
Death	0 (0%)	0 (0%)	1 (6.7%)	5 (35.7%)	

$\chi 2$, Chi square test; MC, Monte Carlo; p, p value for comparing between the studied categories; *, Statistically significant at $p \leq 0.05$

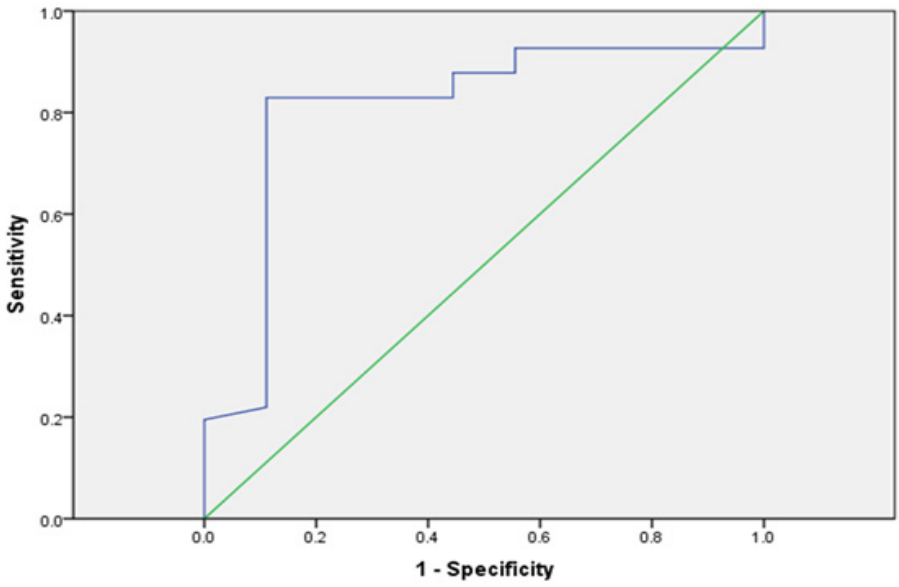

Figure 3. ROC Curve for miRNA 379-5p to Prognosis Patients with Endometrial Cancer (n = 41 vs. 9)

Table 5. Validity (AUC, sensitivity, specificity) for miRNA to Prognosis Patients with Endometrial Cancer According to Stage I , II VS stage III , IV)

	AUC	p	95% C.I	Cut off#	Sensitivity	Specificity	PPV	NPV
miRNA 397-5p	0.809	0.004	0.645 – 0.973	>0.28	82.9	88.9	97.1	46.7

AUC, Area Under a Curve; p value, Probability value; CI, Confidence Intervals; NPV, Negative predictive value; PPV, Positive predictive value; *, Statistically significant at p ≤ 0.05; #, Cut off was choose according to Youden index

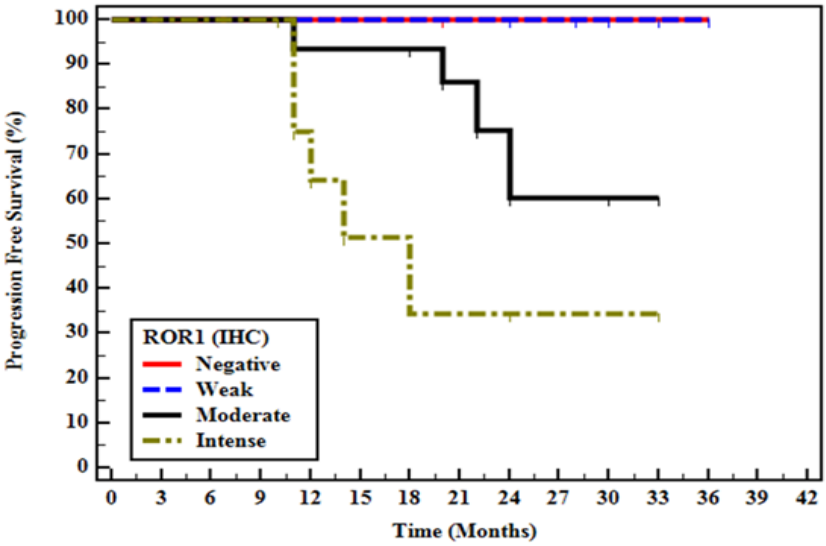

Figure 4. Kaplan Meier Curves of Progression-Free Survival (PFS) Stratified According to ROR1 Immunoexpression.

difference in ROR1 immunohistochemical expression regarding histological type and tumor stage of EC (Table 4).

Statistical analysis of the survival data revealed that shorter OS and PFS were significantly associated with ROR1 immunoexpression (p < 0.001, .0.003 respectively) Figure 4, 5.

Prognostic relevance of miRNA 379-5p and ROR1 expression

Receiver operating characteristic curve (ROC) analysis showed that miR-367-5p could differentiate early (stage I, II) from late stage (stage III, IV) of EC with an AUC of 0.809, P < 0.001. The optimal specificity and sensitivity to recognize early from late EC were (88.9% and 82.9 % with a cutoff level > 0.28) (Table 5, Figure 3). Univariate cox hazard regression analysis for diagnostic factors in EC showed significant differences in stage and miR 397-5p expression in EC (Table 6), suggesting that these parameters are indicators of EC progression.

Discussion

Endometrial cancer incidence rate is highly elevated, and the age of onset is younger than in previous years. Endometrial cancer is still more common in older women, with an increasing mortality rate, although it is also being identified in younger women (Moore and Brewer, 2017). As a result, finding novel validated indicators for early diagnosis as well as therapy targets for EC patients is critical.

Previous research has indicated that miRs play a crucial role in carcinogenesis (Xi et al., 2016), and that miR 379-5p is a tumor suppressor in a variety of malignancies. In hepatocellular carcinoma, for example, miR 379-5p has been shown to increase apoptosis, suppress cell invasion and metastasis, and impede cell cycle progression by targeting the protein tyrosine kinase 2/AKT serine/

Table 6. Univariate and Multivariate COX Regression Analysis for the Different Factors Affecting Overall Survival in Endometrial Cancer Cases (n = 41)

	Univariate		#Multivariate	
	p	HR (95%C.I)	p	HR (95%C.I)
Increase in Grade	0.206	62.613 (0.102 38353.8)		
Increase in Stage	0.014*	9.557 (1.584 – 57.677)	0.056	11.935 (0.935 – 152.35)
Presence of L.N	0.599	4534.9 (0.0 – 2.0×1017)		
ROR1	0.040*	2.384 (1.039 – 5.470)	0.31	2.985 (0.361 – 24.692)
miRNA379-5p	0.021*	0.298 (0.107 – 0.833)	0.597	2.135 (0.128 – 35.604)

HR, Hazard ratio; C.I, Confidence interval; LL, Lower limit; UL, Upper Limit; #,All variables with p<0.05 was included in the multivariate; *, Statistically significant at p ≤ 0.05

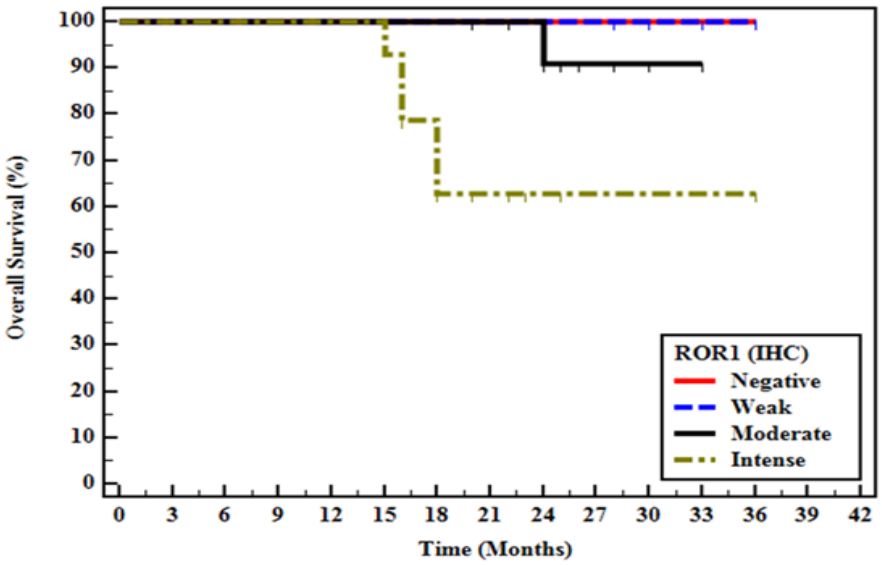

Figure 5. Kaplan Meier Curves of Overall-Free Survival (OS) Stratified According to ROR1 Immunoexpression.

threonine kinase signaling pathway (Chen et al., 2016). In human osteosarcoma cells, overexpression of miR 379 inhibited cell proliferation and colony formation, as well as promoting a G0/G1 cell cycle arrest (Wu et al., 2017). Furthermore, Li et al demonstrated that miR 379 decreased the activity and survival of vascular smooth muscle cells by targeting insulin-like growth factor-1 via extracellular signaling pathways in these cells (Li et al., 2017). These findings show that miR 379 may have a role in a variety of cancers and could be used as a therapeutic target in the treatment of these illnesses.

To our knowledge, this is the first study to investigate the expression patterns of miR-379-5p in endometrial cancer tissue, while Liang et al only looked at its expression in endometrial cell lines in their study (Liang et al., 2021). As a result, the focus of this research was on the expression of miR-379-5p in EC and the relationship between miR-379-5p and its potential target, ROR1. The expression levels of miR-379-5p were shown to be downregulated in endometrial cancer relative to normal surrounding tissue in the current investigation. Reduced miR-379 expression was clearly linked to malignant clinicopathological characteristics in EC patients, such as advanced tumor stage and lymph node metastasis, which was consistent with a previous study by Xu et al., which found that miR-379 expression was significantly downregulated in 96 gastric cancer tissues compared to non-cancerous tissues. Similarly, the expression of miR-379 was drastically reduced in stomach cancer cell lines (Xu et al., 2017).

In addition, Lortet-Tieulent et al. reported that, the suppression of miR-379-5p increased the proliferation, migration, and invasion of endometrial cancer cells; however, overexpression of miR-379-5p inhibited cell proliferation, migration, and invasion in these cells. These findings imply that miR-379-5p works as a tumor suppressor in endometrial cancer and could be studied as a clinically useful therapeutic target (Lortet-Tieulent et al., 2017).

ROR1, a transmembrane protein that belongs to the receptor tyrosine kinase family, is involved in skeletal and brain development (Oishi et al., 1999). However, it is rarely found in adult tissues (Zhou et al., 2017). Although the actual role of ROR1 in cancer is unknown, a growing number of studies have found that ROR1 expression is strongly linked to the development, progression, and metastasis of a variety of human cancers, and that it is associated with aggressive illness and a bad prognosis (Cui et al., 2012; Liu et al., 2020). For example, silencing ROR1 decreased the growth and invasion of ovarian cancer cells (Zhang et al., 2012). ROR1 has been demonstrated to promote lymphoblastic leukemia (Henry et al., 2017), lung cancer (Bicocca et al., 2012), and breast cancer cell survival and proliferation (Nadanaka et al., 2022). ROR1 suppresses melanoma cell invasion but increases epithelial-mesenchymal transition and breast cancer cell metastasis. ROR1 levels beyond a certain threshold were linked to pancreatic cell death (Yamaguchi et al., 2012). This disparity shows that ROR1 may have different functions in different forms of cancer, and its relevance in EC is currently unknown.

ROR1 expression levels in EC were substantially higher than in normal adjacent tissues in our investigation. Upregulated expression ROR1 was associated with malignant clinicopathological characteristics in EC patients, such as advanced tumor stage and lymph node metastasis, which was similar to the results of Abdelbary et al., 2022. This finding also was like that of Zhang et al, who discovered that ROR1 expression is higher in tumour tissues and blood samples of EC patients. ROR1 levels were found to be linked to enhanced Wnt5a and cyclin D1 expression (Zhang et al., 2017) Moreover, Henery et al. discovered that patients with EC have increased ROR1 expression, which promotes tumour growth Henry et al., 2018; Liu et al., 2020). Furthermore, we noticed, the expressions of miR-379-5p and ROR1 mRNA were found to be negatively correlated. This finding was like that of Liang et al., (2021) who discovered that miR-379-

5p targeted and restricted ROR1 expression, and that the effects of miR-379-5p overexpression on endometrial cancer cell proliferation, migration, and invasion were decreased after ROR1 overexpression. These findings revealed that miR379-5p inhibited endometrial cancer cell proliferation, migration, and invasion via decreasing ROR1.

However, the current research was constrained by the lack of investigating the underlying molecular mechanism. As a result, the role of other signaling pathways or associated factors regulated by miR 379- 5p in regulating the proliferation and apoptosis of endometrial cancer cells needs to be examined further, which is what our future research will focus on.

In conclusion, the current study shows that downregulation of miR-379-5p in EC causes ROR1 dysregulation, which may accelerate cancer progression. As a result, our research identifies a new ROR1 regulator and adds to our understanding of the interactions between miR-379 -5p and its EC targets. As a result, our data suggest that miR-379-5p and ROR1 could be useful molecular targets in the treatment of EC.

Author Contribution Statement

Conception: Hala Mosaad, Marwa H.S. Hussien; Interpretation or analysis of data: Mohamed S.H. Ramadan; Preparation of the manuscript: Aziza E. Abdelrahman; Revision for important intellectual content: Ahmed El-azony; Supervision: Mohamed El-Bakry Lashin.

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Ethical Approval

The experimental protocol was approved by the Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request. Compliance with Ethical Standards

Informed consent

Informed consent was obtained from all individual participants included in the study.

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest

The authors declare no conflict of interest.

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