Non-coding RNA Research 6 (2021) 35–41

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: www.sciencedirect.com/journal/non-coding-rna-research

MiRNA 34-a regulate SIRT-1 and Foxo-1 expression in endometriosis

Noha A. Rezk [a], Mohamad Bakry Lashin [b], Norhan A. Sabbah [a,*]

[a] Medical Biochemistry Department, Faculty of Human Medicine, Zagazig University, Zagazig, Egypt
[b] Gynecology & Obstetrics Department, Faculty of Human Medicine, Zagazig University, Zagazig, Egypt

ARTICLE INFO

Keywords:
SIRT1
miR-34a
p53
Bax
Bcl-2 and FoxO-1
Endometriosis

ABSTRACT

Purpose: The role of the Sirutin 1 (SIRT1) and MicroRNA-34 a (miR-34a) in endometriosis and the extent to which the miR-34a/SIRT1/p53 signaling pathway is involved in its pathogenesis is unclear, so we aimed to investigate the expression of miRNA 34-a, SIRT1, Forkhead boxO (FoxO-1), p53 and other apoptotic markers in endometrial tissue of women with endometriosis in order to better understand their role and the mechanisms of their actions in the pathogenesis of such disease and if it is related to apoptosis or not.

Methods: Ectopic and eutopic endometriotic tissues were collected from seventy women with endometriosis while normal endometrial tissues were obtained from 40 fertile women without endometriosis and then gene expression of SIRT-1, miR-34a,p53, Bax, Bcl-2, Bcl-xL and FoxO-1 were measured using RT-PCR.

Results: We detected that SIRT-1 and Bcl-xL genes expressions was significantly up-regulated while miRNA34-a, p53, Bax, Bcl-2 and FoxO-1 were down-regulated in endometrial tissue of endometriotic patients compared to that of those without endometriosis. There was an inverse relationship between SIRT-1a, Bcl-xL genes expressions and miR-34a, p53, Bax, Bcl-2 expressions as well as FoxO-1 expression. These results imply that miR-34a might regulate p53 through SIRT-1 and subsequently FoxO-1 expression in endometriotic tissue, and so it can contribute to the pathogenesis of endometriosis by decreasing the naturally occurring apoptosis in endometrium.

Conclusion: This study may provide a potential biomarker for endometriosis therapeutics. Identification of target genes downstream of these transcriptional factors would allow better understanding of their respective roles in the pathogenesis of endometriosis.

1. Introduction

Endometriosis is defined as chronic inflammatory illness characterized by endometrial tissue outside the uterus, which can shed in response to hormonal changes [1]. The discarded blood falls into neighboring organs, causing swelling and inflammation leading to pain and adhesions [2]. Endometriosis is an important reason of pain and infertility, with a prevalence of 10% in women worldwide [3,4].

Increased proliferation, inflammation, decreased apoptosis, altered cellular immunity, and progesterone resistance are characteristics of endometriosis [5]. Endometriosis is considered as an angiogenic disease, since the ectopic portion needs the formation of extra blood supply, which is a critical part in the lesion's pathogenesis [6].

Sirutin 1 (SIRT1) is a member of the sirtuin family of proteins [7]. It regulates chromatin remodeling, cellular signal and lifespan by its NAD dependent deacetylation action [8]. SIRT1 acts on both histones and non-histone proteins such as p53, forkhead boxO (FoxO) and NF-κB [9]. It has a pivotal role as an oncogenic effect and tumor suppressor [10]. Previous reports reported that SIRT1 played a role in endometrial tumor by targeting sterol regulatory element binding protein 1 (SREBP1) and lipogenesis [11]. Other studies remarked elevated SIRT1 expression in a correlation with poor prognosis of ovarian cancer [12].

MicroRNAs (miRNAs) are non-coding RNA that control gene expression by reducing its translation or decreasing the stability of their target mRNA [13,14]. It has been found that SIRT1 expression and activity are regulated by several miRNAs, miR-34a is one of them [15]. Over-expressing miR-34a decreases SIRT1 protein level and knocking down miR-34a enhances SIRT1 expression [16] P53 has a very complicated relationship with both miR-34a gene and SIRT1 making the miR-34a/SIRT1/p53 signaling pathway of great implication in many diseases including endometriosis [17].

miR-34a was detected as a tumor suppressor gene in neuroblastoma. Its expression was reduced in cancer tissues and in endometriosis patients [18,19].

The pro-apoptotic transcription factor FOXO1 regulates the genes involved in stress responses, longevity, oncogenesis, and is involved in

* Corresponding author.
E-mail addresses: mazenmahmoud17@yahoo.co.uk, NASaeed@medicine.zu.edu.eg (N.A. Sabbah).

https://doi.org/10.1016/j.ncrna.2021.02.002
Received 17 November 2020; Received in revised form 27 January 2021; Accepted 7 February 2021
Available online 13 February 2021

angiogenesis as a negative regulator of blood vessel formation. It has been showed that SIRT1 bind to and deacetylate FoxO1 suppressing its pro apoptotic transcriptional activity [20,21]. The miR-34a/SIRT1/p53 signaling pathway was demonstrated to be turned on in variant conditions and apoptosis of different tissues including the endometrium [17]. The role of the miR-34a in endometriosis and the extent to which the miR-34a/SIRT1/p53 signaling pathway is involved in its pathogenesis is unclear, so we aimed to investigate the expression of miRNA 34-a, SIRT1, FoxO-1, p53 and other apoptotic markers in endometrial tissue of women with endometriosis in order to better understand their role and the mechanisms of their actions in the pathogenesis of such disease and if it is related to apoptosis or not.

2. Subjects and methods

2.1. Subjects

Ectopic and eutopic endometriotic tissues were collected from seventy women with endometriosis undergoing a laparoscopic operation at the Department of Gynecology and Obstetrics, Zagazig University, Egypt. Diagnosis was confirmed by histopathological examination. Cycle stage was evaluated depending on the date of the last menstrual phase and by histological examination, according to Noyes' et al. (1950) criteria [22]. We categorized the endometriosis group as stages I/II/III/IV according to American Fertility Society, 1985 [23].

Normal endometrial tissues were obtained from 40 fertile women without endometriosis undergoing surgery for benign lesion as curettage for cervical erosion. All subjects had regular menstrual cycles and did not receive hormonal treatment for at least 1 month before surgery. We collected the Endometrial samples by curettage, and frozen it immediately in liquid nitrogen, and preserved it at −80 °C until further use. The study was approved by the ethics committee of Zagazig University, and all subjects before inclusion in our study gave a written consent. Body mass index (BMI) was calculated by dividing the weight by the squared value of height in meters. Clinical and demographic characteristics of the studied groups were summarized in (Table 1).

2.2. Methods

2.2.1. Biochemical measurement

Five mL blood was taken and was left for 10 min to clot and afterward was centrifuged. The serum samples were isolated and put away at - 20 °C till the assay. Serum CA125 was estimated by ELISA utilizing the Quantikine human CA125 test kits [R and D Systems, Minneapolis, MN].

2.2.2. RNA extraction including miRNA

For isolation of total RNA according to the manufacturer's instructions of the miRNeasy extraction kit (Qiagen, Valencia, CA) by using 250 μl of QIAzol lysis reagent for 5 min at room temperature. Then, 200 μL of chloroform was added and vortexed for 15 s, incubating the samples for 3 min at room temperature, then centrifuged at 14,000×*g* at 4 °C for 15 min. 600 μL were pipetted in miRNeasy Mini spin column and centrifuged at 8000×*g* for 60 s followed by addition of buffer RWT then buffer RPE before centrifugation at 8000×*g* for 2 min. 20 μL of eluted miRNA was reverse transcribed by incubation for 30 min at 25 °C, 30 min at 42 °C, 5 min at 85 °C, and afterward kept up at 4 °C utilizing the miRNeasy Reverse Transcription Kit (Qiagen, Valencia, CA) as indicated by the manufacturer's instructions.

2.2.3. Quantitative real-time polymerase chain reaction [qRT–PCR]

To assess mRNA expression of glyceraldehyde 3-phosphate dehydrogenase [GAPDH], Foxo 1 and SIRT1, qRT–PCR was performed using SYBR green on a [StratageneTMMx3005P] qPCR instrument [Agilent Technologies]. The PCR conditions for GAPDH, SIRT1 and Foxo1 were as follows: for GAPDH, 35 cycles at 95 °C for 10 s, 64 °C for 10 s and 72 °C for 18 s; for SIRT1, 40 cycles at 95 °C for 10 s, 66 °C for 10 s and 72 °C for 9 s. For FOXO1 gene, 40 cycles of 10 s at 95 °C and 15 s at 60 °C and 72 °C for 9 s. For P53, Bax, Bcl-2 and Bcl-xL, initial denaturation at 94 °C for 5 min, followed by 30 cycles for amplification (94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1.5 min) and 72 °C for 5 min. Primers used are shown in [Table 2]. All PCR reactions were followed using melting curve analysis [24–26].

2.2.4. Real Time-PCR quantification of miRNA-34a

Measurement of miRNA-34a was completed by SYBR green quantitative real-time polymerase chain reaction test utilizing hsa-mir-34a SYBR Green PCR kit (Qiagen, Hilden, Germany) on (StratageneTMMx3005P) qPCR instrument (Agilent Technologies).

The reaction was done on 20 μL final volume containing (2 μL of cDNA, 0.4 μL of hsa-miR-34a-specific primers and 10 μL QuantiTect SYBR Green PCR Master mix) (Qiagen, Hilden, Germany). The sequence of the primers used were 5′-GCGCTGGCAGTGTCTTAGC-3′ forward and 5′-GTGCAGGGTCCGAGGT-3′ reverse. The conditions of the cycling were: denaturation at 95 °C for 3 min then 40 cycles of: 95 °C for 12 s 62 °C for 40 s, and 70 °C for 10 s. At that point, analysis of melting curve was performed, and PCR products were electrophoresed on 2% agarose gels to validate the specific generation of the expected PCR product. miRNA expression level was normalized from that of the internal control RNU1A. The amplitude of miRNA expression change, observed in patients in relation to control group, was analyzed by the ΔCt method [26].

2.3. Statistical analysis

We analyzed our data using a statistical package for the Social Science SPSS 15.0 (Chicago, IL).

Chi square (v2) test was used to interpret the frequencies' differences in distinct groups.

We used mean ± SD to express the serum levels of miRNA. Student's t-test and one-way analysis of variance were used to determine any significant differences among died groups. To assess the correlation among diverse parameters, we used Pearson's correlation. If the P value < 0.05 it was deemed significant. P value was adjusted after Bonferroni correction.

3. Results

3.1. Demographic data of the patients and control groups

The endometriotic patients and control groups did not differ in age, BMI and phase of menstrual cycle but there was a significant difference between patients and controls as regards lipid profile and Ca125 (Table 2).

3.2. Gene expression of the studied parameters in the different groups

We detected that expression of SIRT-1, Bcl-xL in the ectopic and eutopic endometrial samples were over-expressed compared to normal endometrial tissue. SIRT-1, Bcl-xL mRNA level was significantly higher in ectopic endometrial tissues than that of the eutopic tissue in women with endometriosis. On the contrary, miR-34a, FOXO1, P53, bcl2 and Bax expression levels significantly decreased in ectopic and eutopic endometriosis tissue compared controls and in ectopic endometriotic tissues compared to eutopic ones [Table 3].

3.3. Correlation between different parameters expression in endometriosis

We observed that SIRT-1 expression level was inversely correlated with miR-34a, FOXO1, Bcl2, p53 and Bax expression in endometriosis [Figs. 1 and 2] while SIRT-1 expression level was positively correlated with Bcl-xl expression [Table 4]. FOXO1 mRNA expression was positively correlated with miR-34a expression [Fig. 3].

Table 1
Clinical and demographic characteristics of the studied groups.

	Control group [N=40]	Endometriosis group [N=70]	*p*
Age			
≤ 30 [n%]	22 [55]	37 [52.8]	0.61
>30 [n%]	28 [45]	43 [47.2]	
BMI [kg/m^2]	31±4.3	30 ± 4.4	0.67
Waist circumference [cm]	87.3±23.6	96.2±21.5	0.044
Lipid Profile			
Tc [mg/dl]	178.7±20	186.3±58.7	0.043
TG [mg/dl]	101.5±43.8	136.7±47.6	0.04
HDL [mg/dl]	52.6±12.4	39.3±13.6	0,042
LDL [mg/dl]	76.7±28.5	96.6±52.7	0.035
Phase of menstrual cycle			
Early secretory n [%]	9 [22.5]	12 [17.1]	0.70
Mid secretory n [%]	18 [45]	34 [48.6]	
Late secretory n [%]	13 [32.5]	24 [34.3]	
CA 125 [IU/mL]			
≤35 IU/mL [n %]	14 [35]	36 [51.4]	0.041
>35 IU/mL [n %]	36 [65]	34 [48.6]	
AFS stage			
I [n %]		1 [1.4]	
II [n %]		8 [11.4]	
III [n %]		24 [34.4]	
IV [n %]		37 [52.8]	
Gynecological history			
Primary infertility [n%]		25 [35.7]	
Secondary infertility [n%]		20 [28.5]	
Pelvic pain [n%]		64 [91.4]	
Dysmenorrhea [n %]		67 [95.7]	

BMI (Body mass Index),Tc (total cholesterol),TG (triglycerides),HDL(High density lipoprotein), LDL(Low density lipoprotein)

Table 2
Primer sequence and size of amplicon of the studied genes.

	Primer sequence
GAPDH	**Sense** 5′ACCACAGTCCATGCCATCAC 3′ **Antisense** 5′TCCACCACCCTGTTGCTGTA3′
Sirt1	**Sense** 5′ GAGATAACCTTCTGTTCGGTGATGAA3′ **Antisense** 5′ CGGCAATAAATCTTTAAGAATTGTTCG3′
FOXO1	**Sense** 5′-TGG ACA TGC TCA GCA GAC ATC-3′ **Antisense** 5′-TTG GGT CAG GCG GTT CA-3′
P53	**Sense** 5′ TGCGTGTGGAGTATTTGGATG-3′ **Antisense** 5′-TGGTACAGTCAGAGCCAACCTC-3′
Bax	**Sense** 5′ GCC CTT TTC TAC TTT GCC AGC R 3′ **Antisense** 5′TCA GCC CAT CTT CTT CCA GAT3′
Bcl-2	**Sense** 5′ GGC CTT CTT TGA GTT CGG TGG 3′ **Antisense** 5′ GAT AGG CAC CCA GGG TGA TGC3′
Bcl-xL	**Sense** 5′ CTG GTG GTT GAC TTT CTC TCC3′ **Antisense** 5′ GCT GCT GCA TTG TTC CCA TAG3′

Table 3
Expression of the studied parameters in the different group.

	Control [n = 40]	Eutopic EMS goup [n = 70]	Ectopic EMS group [n = 70]
Sirt 1 mRNA expression	0.46 ± 0.21	0.79 ± 0.35[a]	0.98 ± 0.42[a,b]
MiR- 34a expression	0.84 ± 0.23	0.6 ± 0.19[a]	0.45 ± 0.1[a,b]
Foxo1 mRNA expression	0.9 ± 0.21	0.58 ± 0.13[a]	0.24 ± 0.08[a,b]
P53 mRNA expression	0.97 ± 0.23	0.62 ± 0.15[a]	0.31 ± 0.06[a,b]
BAX mRNA expression	0.89 ± 0.0.25	0.65 ± 0.28[a]	0.45 ± 0.13[a,b]
Bcl-2 mRNA expression	0.871 ± 0.37	0.56 ± 0.28[a]	0.23 ± 0.091[a,b]
Bcl-xL mRNA expression	0.81 ± 0.46	1.9 ± 0.94[a]	2.4 ± 0.74[a,b]

[a] $P < 0.05$ when compared to control group.
[b] $P < 0.05$ when compared to eutopic group.

4. Discussion

Endometriosis is a complex and enigmatic gynecologic disease with incompletely understood etiology. The morphologic appearance of endometriosis is marked by proliferation, infiltration, angiogenesis and severe adhesions, retraction, and deformities to the surrounding tissues/organs [27].

The disease has a strong resemblance to neoplastic disease because of the presence of growth factors and cytokines associated with regulating cell proliferation and angiogenesis [28]. In addition, analogous to cancer, endometriosis lesions may be single or present in distant foci, mimicking metastases [29,30].

In the current study, we measured the expression of miRNA 34-a, SIRT1, FoxO-1, p53 and other apoptotic markers expressions in endometrial tissue of women with endometriosis in order to better understand their contribution to pathogenesis of such disease and their mechanisms of actions.

We observed over-expression of SIRT-1 and Bcl-xL in the ectopic and eutopic endometrial samples compared to normal endometrial tissue. SIRT-1 and Bcl-xL mRNA level in the ectopic endometrial tissues of females with endometriosis was significantly higher than that of the eutopic tissue. These results show that SIRT-1 expression may be associated with the progression of endometriosis and this may be by decreasing apoptosis as seen by increased Bcl-xL the anti-apoptotic marker. In this study we measured SIRT1 mRNA level and not its levels in the serum however it is usually correlated.

Yoo et al. (2017) reported that SIRT1 was over-expressed and co-localize in the nuclei of endometrial cells from women with endometriosis and was co associated with BCL6 [31].

SIRT-1 was significantly up-regulated and promoted tumor proliferation, migration, and invasion capacity in endometrial carcinoma [11] as well as in ovarian carcinoma [32].

Sirtuin 1 (SIRT1) regulatory function is mostly related to p53 action. In order to stop the transcription action of p53, it is deacetylated by SIRT1 in a NAD+-dependent way leading to the control of different pathways involved in different ailments and tissue homoeostasis [17].

In our study, the levels of miR-34a expression decreased in ectopic and eutopic endometriosis tissue in relation to its level in the control group without endometriosis. Similarly, Ying, et al. (2017) demonstrated down-regulated miR-34a-5p expression levels in patients with endometriosis [19]. These results support previous findings that miR-34a-5p suppress disease progression, along with diseases associated with the endometrium. miR-34a is one of the most recognized tumor suppressor miRNAs in several tumors which has been reported to be a direct transcriptional target of p53 [33], and Low levels of miR-34a expression had been mentioned in numerous types of cancers such as prostate cancer, hepatocellular carcinoma, chronic lymphocytic leukemia (CLL), colon cancer, and ovarian cancer [34]. Moreover, miR-34a has been found to inhibit mobileular proliferation and invasion in

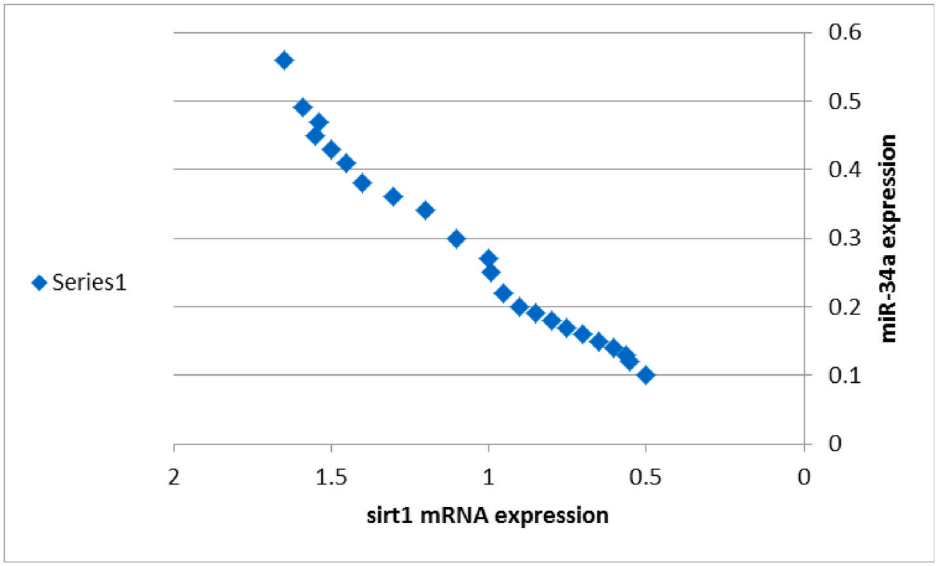

Fig. 1. Negative correlation between sirt1 mRNA expression and miR-34a expression in 70 endometriotic patients.

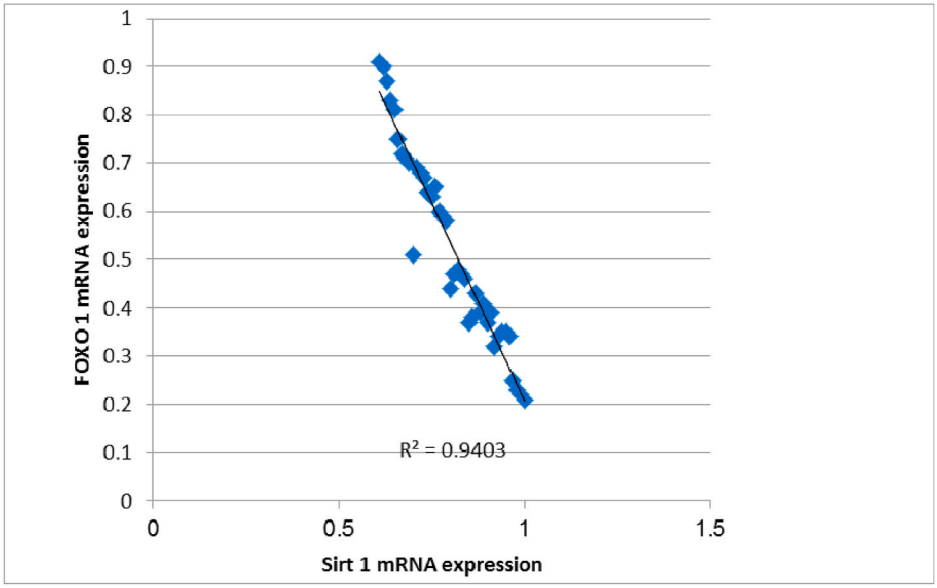

Fig. 2. Negative correlation between sirt1 mRNA expression and FOXO1 expression in 70 endometriotic patients.

Table 4
Correlation between sirt-1 mRNA expression and apoptotic marker.

Apoptotic marker	SIRT-1 mRNA expression	
	r	P
BAX mRNA expression	- 0.87	<0.001
Bcl2 mRNA expression	- 0.73	<0.01
P53 mRNA expression	−0.65	<0.03
Bcl-xl mRNA expression	0.69	<0.035

vitro, which suggested that miR-34a-5p might play a role in inhibiting tumor recurrence [35]. In order to prove the role of miR-34a-5p in tumor suppression we measure the expression of Bcl2, p53 and Bax as pro apoptotic markers and we found their levels was positively corelated with miR-34a-5p, while inversely correlated with SIRT1 which give a huge insight on the pathogenesis of endometriosis and the role of miR-34a-5p and SIRT1in apoptosis or lack of it in endometriosis.

It was stated that the ratio of apoptosis in shed endometrial tissues decreased massively in females suffering from endometriosis [36], proving that there is a big percentage of aging cells in the peritoneal cavity in endometriotic female, meanwhile it was reported that the apoptotic index decreased significantly in glandular epithelium in endometriosis [37].

miR-34a gene is targeted mainly by p53, on the other hand miR-34a can deacetylate most of the acetylation sites of p53, assuming that there is a positive feedback loop in the signaling pathway of miR-34a/SIRT1/p53 in which miR-34a is induced by p53, which is activated by the inhibition of SIRT1which is done by miR-34a explaining their mechanism of action in apoptosis and cell proliferation [17].

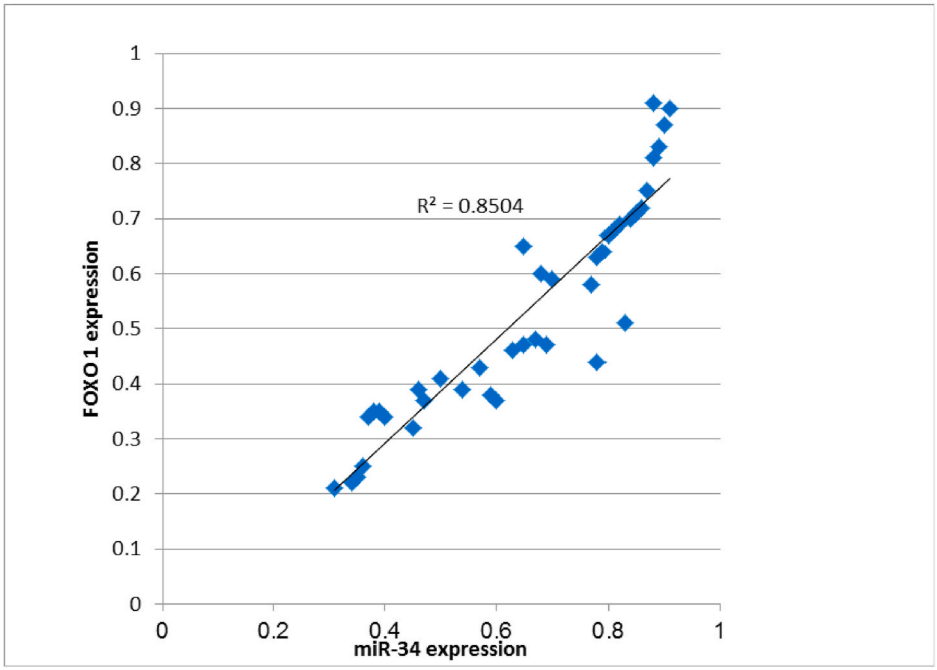

Fig. 3. Positive correlation between miR-34a expression and FOXO1 expression in 70 endometriotic patients.

We additionally measured the expression of the pro-apoptotic transcription factor FOXO1. We found a significantly decreased expression of FOXO-1 in ectopic and eutopic endometrium compared to the control group. In concordance with our results, Shazand et al., 2004, observed a statistically significant decrease of the FOXO1 mRNA in eutopic endometriosis in the secretory phase [38]. These findings in the eutopic endometriosis might be explained by that the unusual gene expression profile of endometrial cells of patients allowed their implantation and survival in an ectopic site, leading to endometriosis lesions. Angiogenesis is an important determinant in tumor initiation, progression, and metastasis. Angiogenesis performs a critical function in endometriosis progression [39]. SIRT1 acts as an essential regulator of post-natal vascular growth therefore, strategies to inhibit SIRT1 activity may also provide a possibility for anti-angiogenesis therapies [40]. SIRT-1 is one of the potential targets of miR-34a [16]. MiR-34a was reported to bind directly to Sirt1 mRNA and regulate cell apoptosis via Sirt1-p53 pathway [16,41]. Sirt1 has recently been implicated as a novel modulator of vascular endothelial cell homeostasis, playing a key role in angiogenesis through the deacetylation of FoxO1 [21,40]. In endothelial cells, silencing of Sirt1 was accompanied by impaired angiogenic function in vitro [42].

Here we showed by correlation analysis that SIRT-1 expression was inversely correlated with miR-34a levels therefore, showed a potential role of miR-34a in inhibiting expression of SIRT-1 in endometriosis.

In our study, SIRT-1 expression level was negatively correlated with FoxO-1. Some observe also that miR-34a modulates downstream targets of SIRT1 such as FoxO-1 an essential negative regulator of blood vessel development [43]. The anti-angiogenesis effect of FOXO1 occurs through its inhibition on angiogenesis-related molecules such as HIF-1α and VEGF [44].

In summary, SIRT-1 was over-expressed and level of miR-34a and FoxO-1 were markedly decreased in endometriosis tissues. These results imply that miR-34a might regulate p53 through SIRT-1 and subsequently FoxO-1 expression in endometriotic tissue, and so it can contribute to the pathogenesis of endometriosis by decreasing the naturally occurring apoptosis in endometrium. This study may provide a potential biomarker for endometriosis therapeutics. Identification of target genes downstream of these transcriptional factors would allow better understanding of their respective roles in the pathogenesis of endometriosis.

Ethical approval

Ethical committee of Zagazig University Faculty of Medicine approved this study (RF 5-719- ZU-09).

CRediT authorship contribution statement

Noha A. Rezk: Supervision, Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Funding acquisition, Data curation, Validation. **Mohamad Bakry Lashin:** Funding acquisition, Formal analysis, Validation, Resources. **Norhan A. Sabbah:** Visualization, Conceptualization, Methodology, Funding acquisition, Investigation, Project administration, Writing - original draft, Writing - review & editing, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

we acknowledged all subjects included in our study.

References

[1] B.D. McKinnon, D. Bertschi, N.A. Bersinger, M.D. Mueller, Inflammation and nerve fiber interaction in endometriotic pain, Trends Endocrinol. Metabol. 26 (1) (2015) 1–10.
[2] S.1 Kennedy, A. Bergqvist, C. Chapron, T. D'Hooghe, G. Dunselman, R. Greb, L. Hummelshoj, A. Prentice, E. Saridogan, ESHRE guideline for the diagnosis and treatment of endometriosis, Hum. Reprod. 20 (10) (2005) 2698–2704, 2005 Oct.
[3] L.C. Giudice, Clinical practice. Endometriosis, N. Engl. J. Med. 362 (25) (2010) 2389–2398.
[4] K.J. Holoch, B.A. Lessey, Endometriosis and infertility, Clin. Obstet. Gynecol. 53 (2) (2010) 429–438.
[5] S.L. Young, B.A. Lessey, Progesterone function in human endome- trium: clinical perspectives, Semin. Reprod. Med. 28 (1) (2010) 5–16, 2010.
[6] P.G. Groothuis, A.W. Nap, E. Winterhager, R. Grümmer, Vascular development in endometriosis, Angiogenesis 8 (2005) 147–156.
[7] R.A. Frye, Characterization of ve human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins [sirtuins] metabolize NAD and may have protein ADP-ribosyltransferase activity, Biochem. Biophys. Res. Commun. 260 (1999) 273–279.
[8] M.C. Haigis, D.A. Sinclair, Biological insights and disease relevance, Mammalian Sirtuins, Annu. Rev. Pathol. 5 (2010) 253–295.
[9] J. Luo, A.Y. Nikolaev, ShI. Imai, D. Chen, F. Su, A. Shiloh, L. Guarente, W. Gu, Negative control of p53 by Sir2alpha promotes cell survival under stress, Cell 107 (2001) 137–148, 27.
[10] N.Y. Song, Y.J. Surh, Janus-faced role of SIRT1 in tumorigenesis, Ann. N. Y. Acad. Sci. 1271 (2012) 10–19.
[11] L. Lin, X. Zheng, C. Qiu, S. Dongol, L.V. Qingtao, J. Jiang, B. Kong, C. Wang, SIRT1 promotes endometrial tumor growth by targeting SREBP1 and lipogenesis, Oncol. Rep. 32 (2014) 2831–2835.
[12] D.H. Mvunta, T. Miyamoto, R. Asaka, Y. Yamada, H. Ando, Sh Higuchi, K. Ida, H. Kashima, T. Shiozawa, Overexpression of SIRT1 is associated with poor outcomes in patients with ovarian carcinoma, Appl. Immunohistochem. Mol. Morphol.: Appl. Immunohistochem. Mol. Morphol. 25 (6) (2017) 415–421. July 2017.
[13] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2) (2004) 281–297, 2004 Jan 23.
[14] Ambros V1, The functions of animal microRNAs, Nature 431 (2004) 350–355. Sep. 16.
[15] M. Yamakuchi, MicroRNA regulation of SIRT, Front. Physiol. 3 (2012) 68.
[16] M. Yamakuchi, M. Ferlito, C.J. Lowen- stein, miR-34a repression of SIRT1 regulates apoptosis, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 13421–13426.
[17] X.-F. Tian, F.-J. Ji, H.-L. Zang, H. Cao, Activation of the miR-34a/SIRT1/p53 signaling pathway contributes to the progress of liver fibrosis via inducing apoptosis in hepatocytes but not in HSCs, PloS One 11 (7) (2016), e0158657, https://doi.org/10.1371/journal.pone.0158657.
[18] C. Welch, Y. Chen, R.L. Stallings, MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblas- toma cells, Oncogene 26 (2007) 5017–5022.
[19] M.A. Ying, H.U.A.N.G. Yu-Xin, C.H.E.N. Yan-Ying, miRNA-34a-5p downregulation of VEGFA in endometrial stem cells contributes to the pathogenesis of endometriosis, Mol. Med. Rep. 16 (2017) 8259–8264.
[20] Y. Yang, H. Hou, E.M. Haller, S.V. Nicosia, W. Bai, Suppression of FOXO1 activity by FHL2 through SIRT1-mediated deacetylation, EMBO J. 24 (5) (2005) 1021–1032. Mar 9.
[21] M. Potente, L. Ghaeni, D. Baldessari, R. Mostoslavsky, L. Rossig, F. Dequiedt, J. Haendeler, M. Mione, E. Dejana, F.W. Alt, A.M. Zeiher, S. Dimmeler, SIRT1 controls endothelial angiogenic functions during vascular growth, Genes Dev. 21 (20) (2007) 2644–2658. Oct 15.
[22] R.W. Noyes, A.T. Hertig, J. Rock, Dating the endometrial biopsy, Fertil. Steril. 1 (1950) 3–9.
[23] American Fertility Society, Revised American fertility society classification of endometriosis, Fertil. Steril. 43 (1985) 351–352.
[24] P. Urbánek, L.-O. Klotz, Posttranscriptional regulation of FOXO expression: microRNAs and beyond, Br. J. Pharmacol. 174 (12) (2017) 1514–1532, 2017 Jun.
[25] G. Karaliotas, K. Mavridis2, A. Scorilas, G.C. Babis, Quantitative analysis of the mRNA expression levels of BCL2 and BAX genes in human osteoarthritis and normal articular cartilage: an investigation into their differential expression, Mol. Med. Rep. 12 (2015) 4514–4521, 2015.
[26] K. Livak, T. Schmittgen, Analysis of relative gene expression data using RealTime quantitative PCR and the 2-ΔΔCt method, Methods 25 (2001) 402–408.
[27] M. Vignali, M. Infantino, R. Matronem, I. Chiodo, E. Somigliana, M. Busacca, Endometriosis: novel etiopathogenetic concepts and clinical perspectives, Fertil. Steril. 78 (2002) 665–678.
[28] P. Bellelis, D.F. Barbeiro, L.V. Rizzo, E.C. Baracat, M.S. Abrão, S. Podgaec, Transcriptional changes in the expression of chemokines related to natural killer and T-regulatory cells in patients with deep infiltrative endometriosis, Fertil. Steril. 99 (2003) 1987–1993.
[29] E.J. Thomas, I.G. Campbell, Evidence that endometriosis behaves in a malignant manner, Gynecol. Obstet. Invest. 50 (2000) 2–10.
[30] M.S. Abrao, S. Podgaec, J.A. Dias Jr., M. Averbach, R. Garry, L.F. Ferraz Silva, F. M. Carvalho, Deeply infiltrating endometriosis affecting the rectum and lymph nodes, Fertil. Steril. 86 (2006) 543–547.
[31] J.Y. Yoo, T.H. Kim, A.T. Fazleabas, et al., Contributes to the pathogenesis of endometriosis and progesterone resistance, KRAS Activation and over-expression of SIRT1/BCL6, Sci. Rep. 7 (1) (2017) 6765.

[32] S. Gonfloni, V. Iannizzotto, E. Maiani, G. Bellusci, S. Ciccone, M. Diederich, P53 and Sirt1: routes of metabolism and genome stability, Biochem. Pharmacol. 92 (2014) 149–156.
[33] G. Misso, M.T. Di Martino, G. De Rosa, A.A. Farooqi, A. Lombardi, V. Campani, M. R. Zarone, A. Gullà, P. Tagliaferri, P. Tassone, M. Caraglia, Mir-34: a new weapon against cancer? Mol. Ther. Nucleic Acids 3 (2014) e194.
[34] X.J. Li, Z.J. Ren, J.H. Tang, MicroRNA-34a: a potential therapeutic target in human cancer, Cell Death Dis. 5 (7) (2014), e1327.
[35] S.J. Walton, A. Lewis, R. Jeffery, H. Thompson, R. Feakins, E. Giannoulatou, C. Yau, J.O. Lindsay, S.K. Clark, A. Silver, Familial adenomatous patients with desmoid tumours show increased expression of miR-34a in serum and high levels in tumours, Oncoscience 3 (2016) 173–185.
[36] H.M. Gebel, D.P. Braun, A. Tambur, D. Frame, N. Rana, D.P. Dmowski, Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis, Fertil. Steril. 9 (1998) 1042–1047.
[37] W.P. Dmowski, J. Ding, J. Shen, N. Rana, B.B. Fernandez, D.P. Braun, Apoptosis in endometrial glandular and stromal cells in women with and without endometriosis, Hum. Reprod. 16 (2001) 1802–1808.
[38] K. Shazand, S. Baban, C. Prive´, B. Malette, P. Croteau, M. Lagace´, J.-B. Racine, P. Hugo, FOXO1 and c-jun transcription factors mRNA are modulated in endometriosis, Mol. Hum. Reprod. 10 (12) (2004) 871–877.
[39] S.H. Kim, Y.M. Choi, H.D. Chae, C.H. Kim, B.M. Kang, Decreased expression of angiogenin in the eutopic endometrium from women with advanced stage endometriosis, J. Kor. Med. Sci. 23 (2008) 802–807.
[40] M. Potente, S. Dimmeler, Emerging roles of SIRT1 in vascular endothelial homeostasis, Cell Cycle 7 (14) (2008) 2117–2122. Landes Bioscience).
[41] M. Yamakuchi, C.J. Lowenstein, MiR-34, SIRT1 and p53: the feedback loop, Cell Cycle 8 (2009) 712–715.
[42] H. Ota, M. Akishita, M. Eto, K. Iijima, M. Kaneki, Y. Ouchi, Sirt1 modulates premature senescence-like phenotype in human endothelial cells, J. Mol. Cell. Cardiol. 43 (2007) 571–579.
[43] T. Zhao, J. Li, A.F. Chen, MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1, Am. J. Physiol. Endocrinol. Metab. 299 (1) (2010) E110–E116.
[44] T. Furuyama, K. Kitayama, Y. Shimoda, M. Ogawa, K. Sone, K. Yoshida-Araki, Abnormal angiogenesis in Foxo1 [Fkhr]-deficient mice, J. Biol. Chem. 279 (2004) 34741–34749.