Original Article

Modulation of Long Non-coding RNA FAS-AS1/FAS/Caspase3 Axis in Endometriosis: A Cross-sectional Study

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Abstract

Background: An increasing number of studies have demonstrated that excessive proliferation and apoptosis play a pivotal role in the development of endometriosis. **Aim:** The aim of the study was to evaluate the expression of long non-coding RNA (lncRNA) FAS-AS1, FAS, soluble Fas (sFas) and caspase-3 in patients with different stages of endometriosis. **Setting and Design:** The design of the study was a cross-sectional study. **Materials and Methods:** The relative expression of lncRNA FAS-AS1 and FAs gene was evaluated by the quantitative real-time polymerase chain reaction in 60 ectopic endometrial samples from women with endometriosis in relation to 85 normal endometrial tissues from healthy women, whereas the protein level of sFAs in the peritoneal fluid samples and cleaved caspase-3 in ectopic and normal endometrial tissue samples were determined using the enzyme-linked immunosorbent assay and western blot, respectively. Furthermore, *in silico* analyses were performed to investigate protein–protein interactions as well as molecular function and cellular location of selected proteins. **Statistical Analysis Used:** The student's *t*-test was used to analyse the difference between the means of the two groups. **Results:** The expression of FAS and sFas increased in endometriosis tissues as compared to the control group ($P < 0.05$). However, lncRNA FAS-AS1 and cleaved caspase-3 decreased in ectopic endometrial tissues compared to normal endometrial tissues and low lncRNA FAS-AS1 expression was correlated with disease stages. In addition, the *in silico* analysis revealed the importance of FAS/caspase3 in the biological processes involved in the development of endometriosis. **Conclusion:** The current study suggests that lncRNA FAS-AS1 may function as an ectopic endometriotic suppressor. Moreover, the results showed that severity of endometriosis is also closely correlated with the expression of lncRNA FAS-AS1 and sFAS.

Keywords: *Endometriosis, enzyme-linked immunosorbent assay, long non-coding RNA, polymerase chain reaction*

Introduction

Endometriosis is a non-cancerous gynaecological disorder distinguished by the extrauterine growth of endometrial-like epithelium and stroma, most commonly in the pelvic peritoneum, ovaries, cul-de-sac and broad or uterosacral ligaments.[1,2] This common gynaecological disease affects 6%–10% of women of reproductive aged.[3] The most common clinical manifestations of endometriosis are dysmenorrhoea, dyspareunia, chronic pelvic pain and infertility, all of which harm the quality of life. Furthermore, approximately 1% of women with endometriosis is undergoing malignant transformation.[4] Some patients, however, are asymptomatic or have mild symptoms. Early menarche, low BMI, Caucasian race,

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age (25–29 years), a small number of births and alcohol consumption are all the risk factors for endometriosis.[5]

Endometriosis and its complications have a complex pathogenesis that involves numerous pathways. Endometriosis theories have included retrograde menstruation,[6] local immunity, coelomic metaplasia and metastatic spread, as well as possible stem cell and genetic origins.[7] Endometriosis is caused by the breakdown of the extracellular matrix, abnormal adhesion, peritoneal invasion and the growth of ectopic endometrial cells.[8] Vascular endothelial growth factor (VEGF), cytochrome P450, oestrogen, progesterone, androgen receptor, as well as the p53 gene, have all been shown over the last decade to be implicated in the initiation, development and progression of endometriosis.[9]

Long non-coding RNAs (LncRNAs) are RNAs longer than 200 nucleotides and do not code for proteins. Their expression level is lower than that of protein-coding genes and more tissue-specific.[10] It is well understood that lncRNAs perform a variety of biological functions such as gene transcription, RNA maturation, protein transport and chromatin remodelling. New research indicates that lncRNAs play roles in endometriosis initiation and progression, including endometrial cell proliferation, migration and invasion.[11,12] Some recent studies have elucidated the critical role of lncRNAs such as H19, AC002454.1 and LINC01279 in the pathobiology of endometriosis.[13,14] Some studies have found a link between abnormal lncRNA expression and endometriosis, as well as the promise of these lncRNAs and miRNAs as diagnostic and prognostic biomarkers.[7,15]

The growth of endometriotic lesions is tightly regulated by the balance between cell proliferation and apoptosis. The abnormal survival ability of endometriotic cells has been associated with the overproduction of antiapoptotic factors and the underproduction of proapoptotic factors.[16] Moreover, apoptosis plays a pivotal role in addressing the immune homeostasis in the peritoneal microenvironment which is important in endometriosis. It was shown that LncRNA modulate apoptosis in the endometrium.[17]

FAS antisense RNA 1 (FAS-AS1) is a long non-coding RNA found at 10q23.31 that is transcribed from the antisense strand of the FAS gene intron 1.[18] lncRNA FAS-AS1 is assumed to regulate FAS splicing and the production of soluble Fas (sFas). Furthermore, by binding to FasL, sFas can protect cells from apoptosis. It has also been reported that the lncRNA FAS-AS1 binds and sequesters the RBM5 protein, preventing RBM5-mediated exon 6 skipping and, as a result, lowering sFas.[19]

When compared to normal endometrial tissue, ectopic endometrium has a higher level of anti-apoptotic factors and a lower level of pro-apoptotic factors. Endometrial cells can thus survive within the peritoneal cavity.[20] It has been demonstrated that the caspase activation mechanism is an important signalling pathway causing apoptosis.[21] Caspase-3 is an important upstream regulator of apoptosis.[22]

Some lncRNAs are implicated in the pathophysiology of endometriosis, but the potential functional roles of lncRNA FAS-AS1 and the FAS gene in endometriosis are still unknown. This study hence aims to investigate the expression pattern and clinical significance of lncRNA FAS-AS1 and FAS in ectopic endometriotic tissue and compare them with normal endometrial tissue of endometriosis-free women to obtain new insights into the pathophysiology of endometriosis.

Materials and Methods

Sampling

This study was conducted on 60 women with pathologically confirmed endometriosis who were admitted to Aria Hospital (Rasht, Iran), from October 2021 to November 2022. All of the participants underwent laparoscopic or laparotomic surgery. During an elective diagnostic laparoscopy for endometriosis, ectopic endometrial tissue samples were obtained. Endometriosis was confirmed by pathological biopsy by two pathologists working independently. Participants were excluded from the study if they had autoimmune diseases, chronic heart/liver diseases or a history of hormonal and systemic steroid therapy. During a diagnostic laparoscopy, 85 normal endometrial (eutopic) tissue samples were collected from women who had subfertility but no evidence of endometriosis. Individuals with leiomyoma, adenomyosis, cancer or a history of endometriosis were excluded from the control group. Disease stages were assigned using the revised American Society for Reproductive Medicine (rASRM) classification. Half of the tissue samples were immediately cryopreserved in liquid nitrogen and stored at −80°C until the molecular analysis was performed and the other half were formalin-fixed and processed for histological analysis. Peritoneal fluid (PF) samples were collected from all the participants under sterile conditions during laparoscopy. All participants were in province of Guilan. After the written consent of all participants was obtained, their complete personal and familial history were recorded. This project was approved by the Graduate Education Council of the Faculty of Sciences of (No. 164059) and conducted as per Declaration of Helsinki.

Real-time polymerase chain reaction

Total RNA was extracted from ectopic (EC) and eutopic (EU) endometrial tissues according to the manufacturer's instructions using Trizol Reagent (*in vitro* gen, USA). The Nanodrop spectrophotometer was used to assess RNA quality and concentration (Thermo Fisher Scientific Inc, Waltham, MA, USA). In addition, RNA integrity was assessed by agarose gel electrophoresis. RNA was reverse-transcribed to cDNA according to the manufacturer's instructions using the M-MuLV Reverse Transcriptase kit (Sinaclon, Iran). The SYBR Blue HS-qPCR Mix Kit was used for quantitative real-time polymerase chain reaction (qRT-PCR) analyses (Sinaclon, Iran). The primers were designed by Oligo 7 (Oligo v7.60, Molecular Biology Insights Inc, Colorado Springs, CO, USA). The following primer sets from 5′ to 3′ were used: GAPDH, (forward, TCGTGGAGTCTACTGGTGTCTTC; reverse, ACTGTGGTCATGAGCCCTTC); FAS, (forward, CGTCCAAAAGTGTTAATGCCCAAG; reverse, CCCTAGCTTTCCTTTCACCTGG) and lncRNA FAS-AS1, (forward, CCACTCAGGCAGCGACTTAC; reverse, CTTCTGGAGGCTTGTTTACCG).

Target sequences were amplified in a total reaction volume of 25 µl containing 20–50 ng DNA, 10 ×PCR buffer (iPLEX GOLD Reagent Kit, Agena Bioscience, Beijing, China), $MgCl_2$ (25 mM), dNTPs (25 mM), 0.5 unit of HotStar Taq DNA polymerase (Agena Bioscience, Beijing, China) and 2 pmol of each primer. The reaction conditions were as follows: 1 min of pre-denaturation at 95°C, 35 cycles of 30s at 95°C and 40s at 60°C. All experiments were carried out in triplicate. The expression levels of lncRNA FAS-AS1 and FAS were normalised to GAPDH as a housekeeping gene. The threshold cycle (Ct) was defined as the fractional cycle number at which the fluorescence exceeded the given threshold. The data obtained from the qRT-PCR were analysed using the ΔΔCt method ($2\ \Delta^{\Delta Ct}$). Each sample was run in triplicate.

Protein extraction and western blotting

Expression of cleaved caspase-3 protein in ectopic and normal endometrial tissue samples was detected by western blot. RIPA buffer (Sigma-Aldrich, Germany) was used for protein extraction from endometrial tissue samples, and total protein was collected by centrifugation at 16,000×g for 15 min. The resulting supernatant was used for the experiment. Protein concentration was determined by the Bradford method with bovine serum albumin as the control. The protein samples were then heated at 100°C for 10 min and equal amounts of protein lysates were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to methanol-activated PVDF membranes (Millipore, Bedford, MA, USA). The blots were blocked in 5% non-fat milk diluted in tris-buffered saline (TBS) containing 0.1% (v/v) Tween-20 (TBS-T) and probed overnight with antibodies against primary antibodies, including, including anti-cleaved caspase-3 antibody (1:5000 dilutions; ab32351, Abcam, UK) and β-actin (1:1000 diluted; A5316, Sigma) at 4°C. After three times of washing with TBS-T buffer, membranes were incubated for 2 h at room temperature in a blocking buffer with secondary antibodies conjugated to horseradish peroxidase (1:5000, Amersham Biosciences, USA). The protein bands were quantified and the mean densities of pixels in the protein bands were measured in ImageJ. Relative light densities of the protein bands were calculated and expressed as a ratio to β-actin.

Enzyme-linked immunosorbent assay

The PF samples were centrifuged (400 g, 7 min, 4°C) to remove minor blood contamination. The supernatant was divided into 0.5 mL aliquots and stored at − 80°C until needed. The concentration of sFAS was determined using enzyme-linked immunosorbent assay kits (Abcam, UK) and the manufacturer's instructions. In brief, the supernatant was incubated with a reaction solution, followed by a stop solution. Finally, optical density (OD) values were measured at 450 nm using a microplate reader to assess sFas expression levels.

Identification of predicted long non-coding RNAs of FAS

To identify predicted lncRNAs that affect FAS, four different databases were used, including NPInter (http://bigdata.ibp.ac.cn/npinter4; Institute of Biophysics, Chinese Academy of Sciences; version 4.0; accessed on 11 May 2023), RNAInter (http://www.rnainter.org/; Department of Bioinformatics, Southern Medical University; version 4.0; accessed on 11 May 2023), LncRNADisease (http://www.rnanut.net/lncrnadisease/; Department of Biomedical Informatics, Peking University Health Science Center, China; version 2.0; accessed on 11 May 2023) and RAID (https://www.rna-society.org/raid2/; College of Bioinformatics Science and Technology, Harbin Medical University; version 2.0; accessed on 11 May 2023). We looked for lncRNAs that were common among all four databases. The regulatory link between lncRNAs and FAS was visualised using Cytoscape software (version 3.9.2).

Construction of the protein–protein interactive network

The STRING database (search tool for the retrieval of interacting genes/proteins) is a biological database and web resource that can search for all known and predicted interactions between proteins, including

physical interactions and functional associations, and then generate a protein–protein interactive (PPI) network that includes all of these proteins and all of their interactions. STRING app (version 11.5, https://string-db.org/) in Cytoscape (version 3.9.2) predicted PPI for FAS and CASP3 based on evidence sources such as text mining, experimental evidence, databases, co-expression, neighbourhood, gene fusion and co-occurrence. STRING: Protein query was used as the data source to discover the relationships between FAS and CASP3, as well as the first 20 associated genes. *Homo Sapiens* was selected as an organism. Only nodes with a confidence score >0.400 were included in the network and the edge score was calculated based on molecular action. The Cytoscape platform was employed to develop, design and organise the network.

Functional and pathway enrichment analysis

A functional gene enrichment analysis was conducted on the FAS/CASP3 network to explore their possible biological functions using the STRING Enrichment tool (version 2.0.0) within the Cytoscape platform. The analysis included several functional enrichments relevant to the laboratory data, but no specific false discovery rate values were used.

Statistical analysis

Data for continuous variables were expressed as the mean ± standard deviation $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed in GraphPad Prism 8 (GraphPad Software, CA, USA) and SPSS-21 (IBM Corp., Armonk, NY, USA) for Windows. All the networks were analysed based on relevant databases or online data analysis tools.

Results

Table 1 summarises the general characteristics and clinicopathological features of 85 control participants and 60 endometriosis patients. The results showed no significant difference in the mean age between the endometriosis patients (32.50 ± 1.39) and the control group (33.05 ± 0.94) ($P = 0.15$). Fifty-three (88.33%) patients complained of dysmenorrhoea and 37 (61.66%) had dyspareunia. Among the controls, 13 (15.29%) reported dysmenorrhoea and 8 (9.41%) reported dyspareunia. According to the rASRM classification, 31.66% ($n = 19$) of the women had minimal or mild endometriosis (stages I/II), while 68.33% ($n = 41$) had moderate or severe endometriosis (stages III–IV).

Since Fas is a key regulator of apoptosis and a target of lncRNA FAS-AS1, real-time PCR was employed to measure lncRNA FAS-AS1 in ectopic endometrial tissue and normal endometrium [Figure 1a]. The

Table 1: General characteristics and clinicopathological features of endometriosis patients and control subjects

Parameter	Endometriosis (n=60), n (%)	Controls, (n=85), n (%)	P
Age (years) ± SD	32.50±1.39	33.05±0.94	0.15
BMI (kg/m^2)	22.1±1.05	23.3±1.8	0.29
Symptoms			
Chronic pelvic pain	51 (85)	11 (12.94)	<0.0001
Dysmenorrhea	53 (88.33)	13 (15.29)	<0.0001
Dyspareunia	37 (61.66)	8 (9.41)	<0.0001
Cycle length (day)	30.5±3.1	31.3±2.0	0.89
Spontaneous abortion	7 (11.66)	6 (7.08)	0.25
rASRM classification			
Stage I/II	19 (31.66)	-	-
Stage III/IV	41 (68.33)	-	-

Stage I=Minimal disease, Stage II=Mild disease, Stage III=Moderate disease, Stage IV=Severe disease, rASRM=Revised American Society for Reproductive Medicine, SD=Standard deviation, BMI=Body mass index

results showed that lncRNA FAS-AS1 expression was significantly lower in endometriosis tissue samples compared to the control group (3.95 ± 0.16 vs. 4.49 ± 0.36, $P < 0.0001$). The differences in lncRNA FAS-AS1 expression levels between patients at different stages of disease were investigated further [Figure 1b] and it was discovered that lncRNA FAS-AS1 was expressed significantly lower in stage III/IV compared to stage I/II (3.92 ± 0.12 vs. 4.03 ± 0.14, $P = 0.001$). FAS levels in ectopic endometrial tissues were significantly higher than in normal endometrial tissue samples, as shown in Figure 1c ($P < 0.0001$). However, there was no significant difference in FAS gene expression between early-stage (stage I/II) and advanced-stage disease (stage III/IV) ($P = 0.87$), as mentioned in Figure 1d.

Analysis of sFas protein in PF samples revealed that the median concentration levels of the protein in endometriosis and controls were 875.3 pg/ml and 562.8 pg/ml, respectively ($P < 0.0001$) [Figure 2a]. In addition, the expression of sFAS protein is higher in patients with endometriosis at advanced stage [Figure 2b]. Western blotting results showed that the relative levels of cleaved caspase-3 in patients were significantly lower than that in controls (0.73 ± 0.08 vs 0.83 ± 0.02, $P = 0.005$) [Figure 3a and c]. As mentioned in Figure 3b, the median relative levels of cleaved caspase-3 in patients with stage I/II disease and patients with stage III/IV disease were 0.79 and 0.76, respectively ($P = 0.29$).

Cytoscape is a versatile, extensible platform with several plugins that add new visualisation tools and expand the program's network research capabilities. Cytoscape makes it simple to access a network's graphical

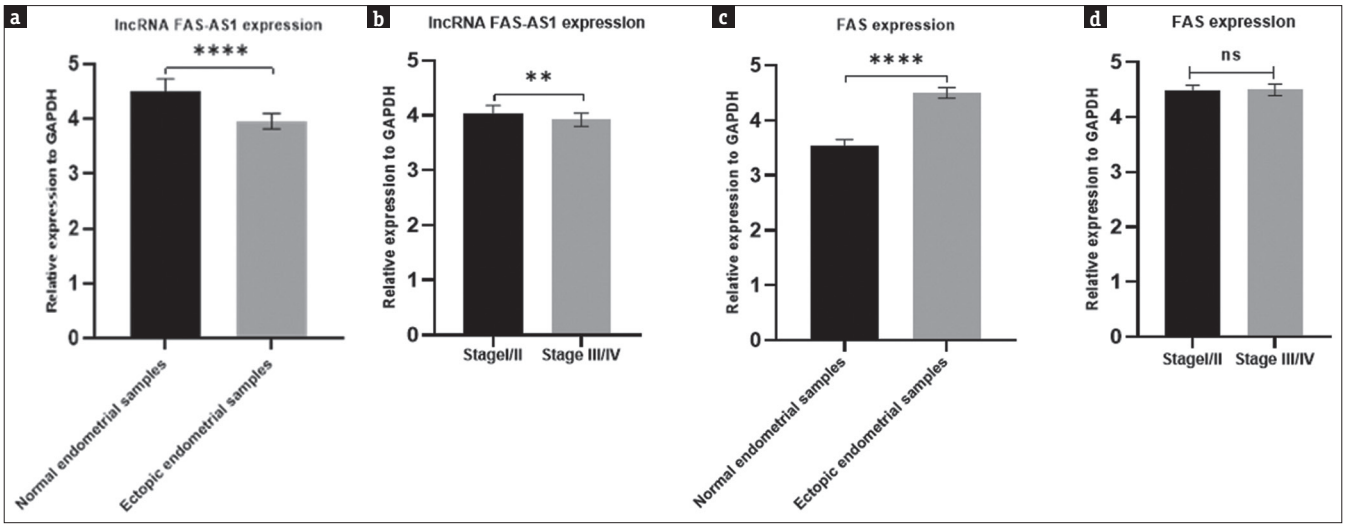


Figure 1: Comparison of long non-coding RNA (lncRNA) FAS-AS and FAS expression in normal endometrium and ectopic endometrial tissue samples. (a) The relative expression of lncRNA FAS-AS1 is downregulated in ectopic endometrial tissues relative to normal endometrial tissues. (b) The relative expression of lncRNA FAS-AS1 is lower in patients with endometriosis at advanced stage. (c) The relative expression of FAS gene is upregulated in ectopic endometrial samples versus normal endometrial samples. (d) The relative FAS expression level in ectopic endometrial tissues according to disease stages. Unpaired *t*-test was used to determine statistical significance. ****$P < 0.0001$, **$P < 0.001$, ns: not significant

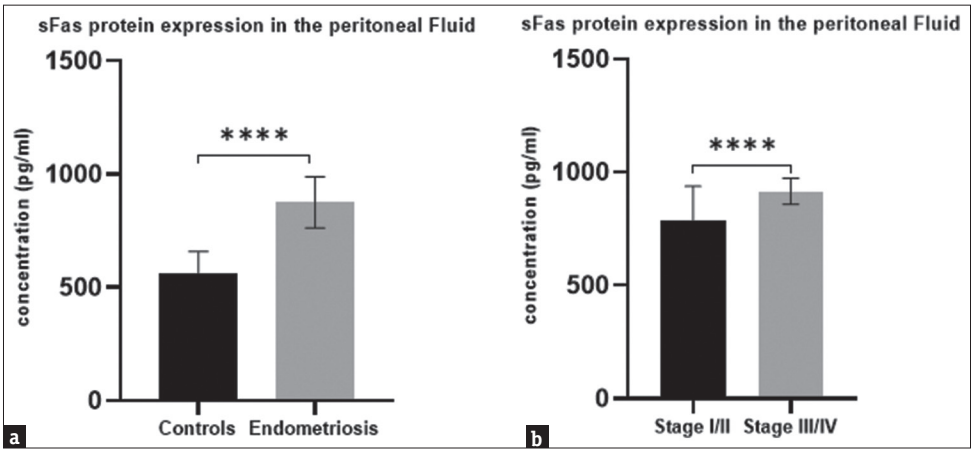


Figure 2: The protein levels of soluble Fas (sFAS) in peritoneal fluid as determine by enzyme-linked immunosorbent assay. (a) The expression of sFAS protein is upregulated in endometriosis versus controls. (b) The expression of sFAS protein is higher in patients with endometriosis at advanced stage. Unpaired *t*-test was used to determine statistical significance. ****$P < 0.0001$

representation and annotate the interactome with various types of data, such as results from large-scale, genome-wide research and information on individual proteins' functions. Using four different databases, the lncRNAs that affect FAS were identified and visualised in the Cytoscape platform as a lncRNA-Protein/mRNA network. All databases predicted a total of 15 lncRNAs in common [Figure 4a]. Depending on the context, each of these lncRNAs can alter FAS expression.

The STRING database predicted 216 edges [Figure 4b] with a confidence score of > 0.400 in the PPI network of FAS, CASP3 and the first 20 proteins that associate with, based on various evidence sources such as text mining, experimental evidence, databases, co-expression, neighbourhood, gene fusion and co-occurrence. Protein interactions with a STRING score of <0.4 were excluded from the study due to their low confidence in the interaction. The nodes in the network represented proteins, which were collapsed to represent all proteins produced by a single gene locus. The edges represented protein–protein interactions, indicating that the proteins shared functions. The more lines there are, the more valid the associations and line thickness indicates the strength of evidence for a predicted interaction. In the network, we have highlighted the FAS and CASP3.

In addition, by STRING enrichment analysis in the Cytoscape, enriched biological processes associated with FAS/CASP3 network were identified. Considering the research purpose, some GO biological processes (Immune system process and Regulation

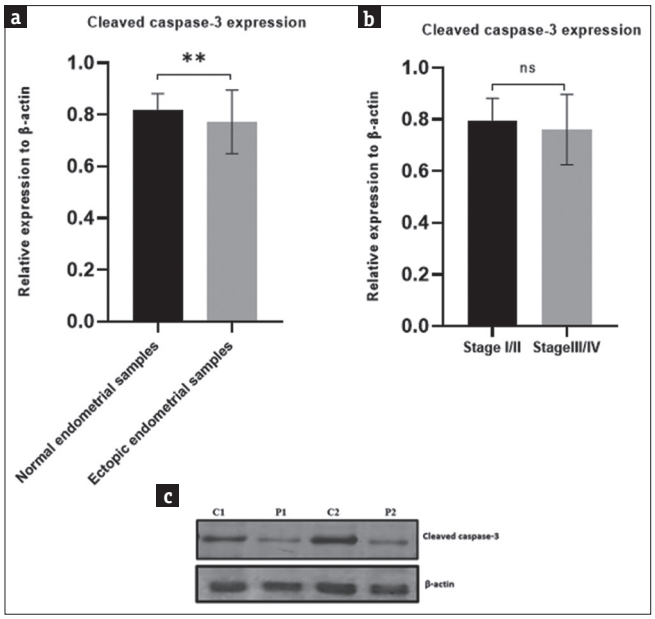


Figure 3: Expression of cleaved caspase-3 by western blot in tissue samples. (a) Relative cleaved caspase-3 expression in normal and ectopic endometrial samples. (b) Relative cleaved caspase-3 expression in different stages of endometriosis. (c) Western blot analysis of normal endometrial tissues (C1, C2) and ectopic samples (P1, stage I/II; P2, stage III/IV). *P* values (**, $P = 0.005$; ns, $P = 0.29$) were determined via unpaired *t*-test

of apoptotic process), KEGG pathways (Apoptosis, p53 signalling pathway and Pathways in cancer), Reactome Pathways (Programmed Cell Death), WikiPathways (VEGFA-VEGFR2 signalling pathway) and TISSUES (Cervical carcinoma cell) databases were selected to evaluate molecular functions and cellular locations [Figure 4c].

Discussion

This study evaluated the expression pattern of lncRNA FAS-AS1, FAS gene and sFas protein in 60 endometriosis patients and 85 controls. The data indicated that the expression of FAS and sFas in ectopic endometrial tissue samples was significantly higher than in controls, which is consistent with previous reports.[23] However, lncRNA FAS-AS1 and cleaved caspase-3 were found to be lower in ectopic endometrial tissues than in normal endometrial tissues. Furthermore, in PF samples, low levels of lncRNA FAS AS-1 and high sFas protein expression were associated with severity of endometriosis.

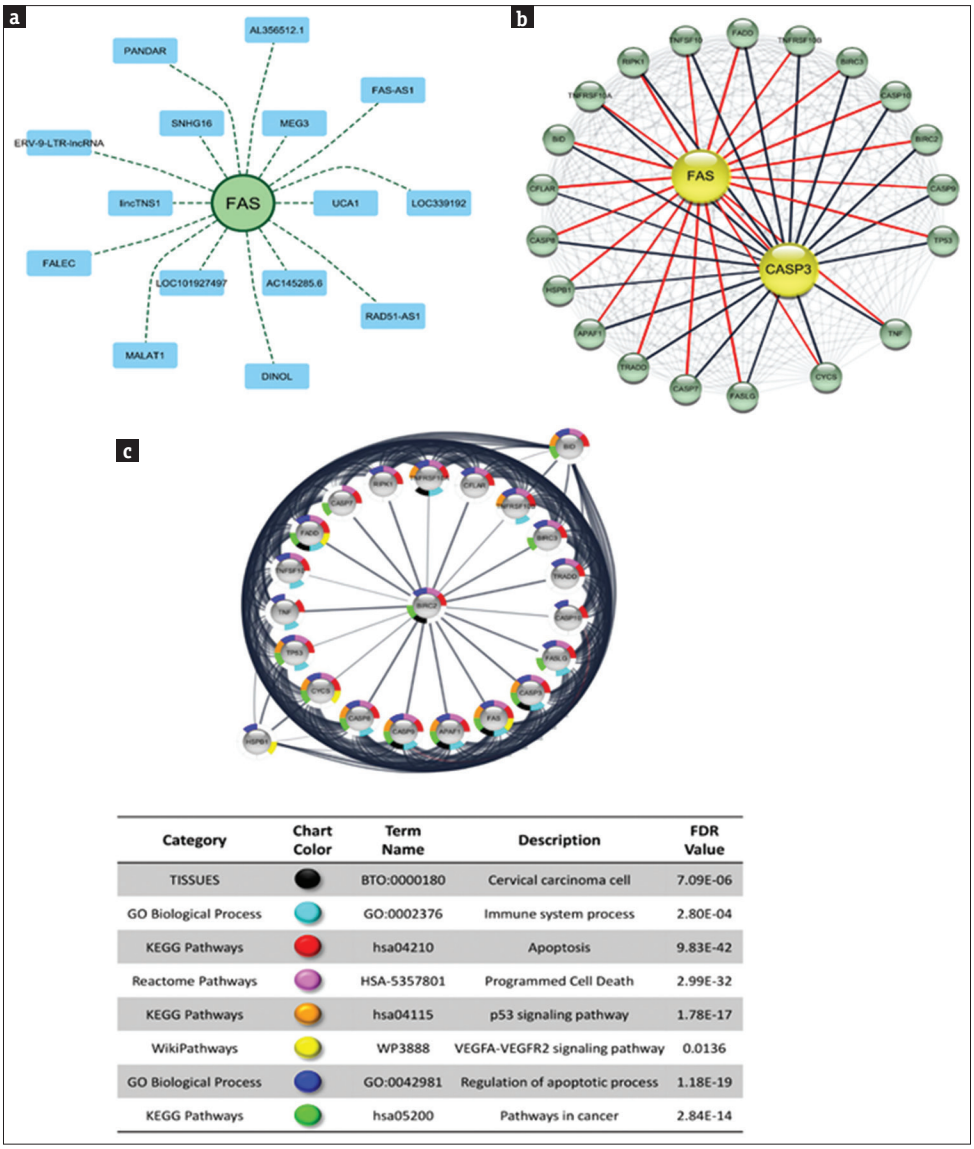
It has been reported that high levels of lncRNA FAS-AS1 inhibit the production of sFas. The levels of sFas mRNA correlate inversely with production of mFas mRNA and mFas protein levels.[19] Moreover, mFas is an effective trigger of apoptosis. Caspase-3 has been proposed to be involved in regulating the growth-promoting properties of ectopic endometrial cells.[24] The findings clearly demonstrated that cleaved caspase-3 was significantly down-regulated in endometriosis.

Growing evidence suggests that lncRNAs can influence gene expression in a variety of ways, including directly binding to mRNA and acting as decoys for microRNAs and proteins.[25] LncRNAs have multiple mechanisms of action and play an important role in various cellular processes related to the development and progression of endometriosis. Differences in the expression of lncRNAs between eutopic and ectopic endometrium could lead to the identification and validation of diagnostic biomarkers, as well as a better understanding of endometriosis physiopathology. Changes in many genes have been shown to occur in endometriosis.[26]

Although studies on lncRNA expression support the hypothesis that lncRNAs play a role in endometriosis, the role of aberrant lncRNA expression in the development of endometriosis has not been thoroughly investigated. A recent study found that the lncRNA HOTAIR increased PRRG4 expression by sponging miR-519b-3p to promote endometrial stromal cell invasion and migration. They suggested that the lncRNA HOTAIR/miR-519b-3p/PRRG4 axis was involved in endometriosis development.[27] Furthermore, NEAT1 silencing was previously shown to inhibit ectopic endometrial stromal cell proliferation, migration and invasion, but it promoted apoptosis by targeting miR-124-3p.[28]

Several apoptosis-related gene expression studies were carried out on endometriotic cells. Delbandi *et al*. found higher gene expression levels of Bcl-2 and Bcl-xl in ectopic endometrial stromal cells compared to eutopic endometrial stromal cells in endometriosis patients.[16]

The lncRNA FAS-AS1 and its target gene FAS, which codes for a protein involved in apoptosis, were examined for altered expression in normal and ectopic endometrial samples. Previous studies have shown that low FAS-AS1 expression can promote the development of diseases such as cancer by inhibiting apoptosis, inducing cell proliferation and mediating immune system escape.[29,30] The expression of lncRNA FAS-AS1 in ectopic endometrial tissue was significantly lower in women with endometriosis than in the control group. A few studies have so far investigated the relationship between FAS-AS1 expression level and endometriosis risk. FAS mRNA expression was found to be upregulated in endometriosis, implying that FAS-AS1 may play a role in the development of endometriosis by regulating FAS-induced apoptosis. Sturlese *et al*. found no variation in the percentage of membrane protein (mFas) bearing mononuclear cells in PF samples of 80 endometriotic



Category	Chart Color	Term Name	Description	FDR Value
TISSUES		BTO:0000180	Cervical carcinoma cell	7.09E-06
GO Biological Process		GO:0002376	Immune system process	2.80E-04
KEGG Pathways		hsa04210	Apoptosis	9.83E-42
Reactome Pathways		HSA-5357801	Programmed Cell Death	2.99E-32
KEGG Pathways		hsa04115	p53 signaling pathway	1.78E-17
WikiPathways		WP3888	VEGFA-VEGFR2 signaling pathway	0.0136
GO Biological Process		GO:0042981	Regulation of apoptotic process	1.18E-19
KEGG Pathways		hsa05200	Pathways in cancer	2.84E-14

Figure 4: *In silico* analysis. (a) Visualisation of long non-coding RNAs that target FAS. (b) PPI network of FAS, CASP3 as predicted by STRING. Each node represents a protein and Edges indicate protein–protein interactions. Yellow nodes represent FAS and CASP3. Bold black edges indicate direct CASP3 connections and red edges indicate FAS connections (Cutoff > 0.40). (c) The functional enrichment analysis of selected genes. Different shaded parts of the circles refer to the related biological processes. All biological processes and pathways had a false discovery rate value < 0.05

women. Furthermore, their findings revealed that endometriosis patients have high and stable levels of Fas mRNA and protein.[23] In contrast, Sbracia *et al*. found low Fas expression and high Fas ligand expression in the ectopic endometrium using immunostaining.[31] The study results showed that sFas expression levels were significantly higher in the PF of women with endometriosis than in controls. Moreover, increased expression of sFas was positively associated with the disease stage. A similar trend was previously observed in lymphoma, where a low level of FAS-AS1 was negatively correlated with the level of sFas expression.[19]

This study also attempted to focus on caspase-3, a downstream factor of Fas involved in programmed cell death. Caspase-3 has been proposed to be involved in regulating the growth-promoting properties of ectopic endometrial cells.[24] The findings clearly demonstrated that cleaved caspase-3 was significantly down-regulated in endometriosis. It has been proposed that the therapeutic effects of rosiglitazone, a PPAR agonist, on endometriosis are related to apoptosis in ectopic endometrial cells mediated by caspase 3 protein activation.[32] Furthermore, increased levels of FOXO1, FOXO3, Bim, procaspase 3, active caspase 3, p53 and p21 have been associated with propofol's therapeutic effect in inducing apoptosis in endometriosis cell line CRL 7566.[33] In contrast to this finding, Delbandi *et al*. demonstrated that caspase-3 expression was lower in eutopic endometrial stromal cells compared

to non-endometriotic controls and ectopic endometrial stromal cells.[16]

Many lncRNAs (such as MALAT3, UCA1, MEG3, SNHG16, PANDAR and FALEC) were found to have an effect on FAS expression based on the *in silico* analysis. FAS-AS1 was also predicted to be one of the lncRNAs that affect FAS. Furthermore, the protein network of the first proteins related to FAS and CASP3 and the enrichment of this network revealed a direct connection between these two proteins and common processes such as apoptosis, programmed cell death, cancer pathways such as cervical cancer, P53 signalling pathway, as well as immune system processes that the effect of each of these paths on the destructive processes of the uterus has been clearly identified. In addition, FAS is also involved in the VEGFA-VEGFR2 signalling pathway separately. The formation of new blood vessels is required for the growth and survival of endometrial cells outside the uterus in endometriosis. VEGFA, a protein that promotes angiogenesis, is overexpressed in endometriotic lesions and VEGFR2, a VEGFA receptor expressed on endothelial cells, is activated to initiate angiogenesis.[34]

The present study faced some limitations. This study only focused on the lncRNA FAS-AS1 and one of its targets, FAS. A single lncRNA may target some genes and a single gene may be regulated by several regulatory RNAs such as microRNAs (miRs) and circular RNAs (circRNAs); thus, other lncRNAs and their targeted genes may contribute to the development of endometriosis.

Conclusion

The results of the present study demonstrated that lncRNA FAS-AS1 was downregulated in endometriosis. Moreover, the results showed that severity of endometriosis are also closely correlated with the expression of lncRNA FAS-AS1 and sFAS.

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Author's contribution

AHS: Methodology, investigation, data collection; ZS: Supervision, study conception, design, conceptualisation, interpretation of the results and draft manuscript writing; FM: Design, analysis, and manuscript review; ZZ: Data analysis, Methodology.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data are available with the corresponding author and willing to share it on request.

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