Abnormal activation of the sonic hedgehog signaling pathway in endometriosis and its diagnostic potency

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Objective: To investigate the abnormal expression of sonic hedgehog (SHH) signaling molecules in 52 eutopic endometrial tissues and its diagnostic potency in endometriosis.
Design: Retrospective study.
Setting: University hospital.
Patient(s): Twenty-six women with histologically confirmed endometriosis and 26 women with histologically normal endometria who were undergoing curettage or hysterectomy were selected.
Intervention(s): None.
Main Outcome Measure(s): The mRNA and protein levels of molecules in the SHH signaling pathway.
Result(s): The levels of *SHH*, smoothened, GLI family zinc finger 3, and its downstream signaling transcription factor (*GLI1*) not only were upregulated in the eutopic endometrium of endometriosis compared with the control endometrium, but also independently predicted the onset and severity of the disease.
Conclusion(s): This study is the first to reveal differences in the activation of the SHH signaling pathway between women with and without endometriosis and suggests that the SHH signaling pathway has potential in the diagnosis of endometriosis. (Fertil Steril® 2018;110:128–36. ©2018 by American Society for Reproductive Medicine.)
This abstract is available in Spanish at the end of the article.

Key Words: endometriosis, sonic hedgehog, signaling pathway

Endometriosis is characterized by the presence of endometrium-like tissues outside of the uterus leading to pelvic pain, dysmenorrhea and infertility (1). Surgeries due to endometriosis constitute the second largest number of surgeries in premenopausal women. Over the past few decades, endometriosis has been actively and extensively investigated, yet the pathogenesis of endometriosis is largely elusive. Endometriosis was first described by Von Rokitansky in 1860 (2). Then, Sampson proposed the most prevalent theory, namely, retrograde menstruation in 1921 (3). Several other accepted theories included metaplasia from müllerian remnants and distant implantation of menstrual debris (4). At the same time, the eutopic endometria of endometriosis have been reported to facilitate a series of metabolic and molecular abnormalities that include an increase in proliferation and angiogenesis and a decrease in apoptosis, thereby allowing the local production of estrogens and generating progesterone resistance (5). These hypotheses have received widespread attention since they can explain some clinical phenomena of endometriosis; however, arguments against these

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hypotheses are abundant, especially for Sampson's retrograde menstruation theory, which has been highly questioned and challenged. Because endometrial fragment reflux into the peritoneal cavity occurs in 90% of women and because only 10% of women have endometriosis (6), other causative factors likely play roles in the development and progression of the disease.

Sonic hedgehog (*SHH*) is one of a group of mammalian hedgehog (*HH*) proteins (*SHH*, Indian hedgehog, Desert hedgehog) that share a common signaling pathway (7). Ligand binding to the *SHH* receptor, Patched1, activates the *SHH* pathway by relieving Patched1-dependent inhibition of Smoothened (*SMO*). Subsequently, suppressor of fused (*SUFU*) and the GLI family zinc fingers (*GLI2*, *GLI3*) transduce the activation signal into the nucleus to regulate the expression of target genes, such as *GLI1* and vascular endothelial growth factor. Aberrant activation of the SHH signaling pathway plays an oncogenic role in various types of gynecological cancers (8,9). SHH signaling is closely linked to oncogenesis through its involvement in enhancing cell proliferation, stem cell maintenance, and cell differentiation and promoting angiogenesis (10–12), highlighting the multiple pathogenic roles of SHH signaling in gynecological disease. For instance, one study demonstrated that down-regulated *KLF9* was positively associated with ectopic lesion establishment in a mouse model of endometriosis through activated Notch and HH signaling (13). Matsumoto et al. (14) confirmed that *HH* proteins were expressed in the mouse uterus and that recombinant *SHH* protein could promote the proliferation of mouse endometrial mesenchyme cells in vitro. However, to date, the precise relationship between endometriosis and the SHH pathway has not been fully established. Thus, we aimed to explore whether the abnormal activation of SHH signaling within the eutopic endometrium in endometriosis patients was relevant to the etiology of endometriosis.

In recent years, an elevated preoperative serum cancer antigen 125 (CA125) level has been used as a non-invasive marker for endometriosis. However, the specificity of CA125 level alone is limited (15). Laparoscopy remains the gold standard for diagnosing endometriosis. Since laparoscopy requires anesthesia and an operation theater, most patients decline this diagnostic protocol. Thus, coupled with a lack of specific diagnostic laboratory biomarkers for endometriosis, these factors lead to a mean latency of 8–11 years from the establishment of endometriosis to a clinical diagnosis (16). These diagnostic delays may have serious consequences in terms of disease progression and may have a profound economic impact (17). However, studies on the identification and functional characterization of SHH signaling in gynecological diseases, especially endometriosis, are limited. Thus, we determined whether four of the SHH pathway molecules could discriminate endometriosis and its associated clinical features. The endometriosis score was evaluated to grade endometriosis into different stages (I-IV) according to the revised American Fertility Society (AFS) criteria (18). Based on the inflammatory response in endometriosis, SHH signaling components were examined with the endometriosis score, mean platelet volume and peripheral marker neutrophil/lymphocyte ratio to determine the diagnostic potential of these components in endometriosis (19).

MATERIALS AND METHODS

Patient and Clinical Information

The samples and clinicopathologic data were collected between 2014 and 2017 from the Department of Gynecology at the First Affiliated Hospital of Harbin Medical University (Harbin, PR China). Institutional Review Board approval for this project was provided by the Ethics Committee of Harbin Medical University (2017117). All methods were performed in accordance with the approved guidelines and regulations. Written informed consent in the study was obtained from each subject. To compare the mRNA and protein expression levels, 26 women with pathological reports of confirmed endometriosis and 26 endometriosis-free patients were enrolled in this study. Each patient's preoperative complete blood count was recorded, and the endometriosis score was assessed (20). None of the patients had received any hormonal therapy within six months before surgery. Women suffering from cancer, a benign ovarian cyst other than an endometrioma, severe pelvic inflammation during surgery, or a known endometrial polyp were excluded from this study.

Twenty-six women (mean age 36 y; range 25–45 y) had histologically confirmed endometriosis. Twenty-six women (mean age 33 y; range 28–36 y) undergoing laparoscopy for idiopathic infertility served as controls in the First Affiliated Hospital of Harbin Medical University. All subjects in the control group were negative for endometriosis. Among all 52 patients, 28 were in the proliferation phase of the menstrual cycle (73%), and14 were in the secretory phase of the menstrual cycle (27%). Twenty-eight (54%) had infertility. In addition, 13 endometriosis patients (25%) presented with progressive dysmenorrhea. The detailed clinical characteristics of the cohort are summarized in Supplemental Figure 1.

Reverse Transcription and Quantitative Real-Time Polymerase Chain Reaction

Eutopic endometrial samples (26 endometriosis samples and 26 controls) were frozen in liquid nitrogen until total RNA extraction using the TRIzol reagent (Invitrogen, America). Due to the current lack of stable standard curves for SHH signaling components in tissues, we applied a double standard curve method. Standard samples were obtained using plasmid (*SHH*) and cDNA (*SMO*, *GLI1* and *GLI3*) templates, with GAPDH standard internal controls. The total RNA was used only if the A260/280 ratio of the absorbances ranged between 1.8 and 2.2 as determined by spectrophotometry. cDNA synthesis was performed in a 20 μl SYBR reaction system with the SYBR premix ExTaqTM II kit (TAKARA, Japan). The standard curves of these selected mRNAs had a good amplification efficiency, R2 and slope, indicating that the method was suitable for mRNA quantification. The mRNA expression levels in the tissues were quantified by establishing standard curves with a set of serially diluted

standard samples. Finally, we validated the standard curves within the mRNA samples, and almost all detectable signals of the samples were on the standard curves, indicating that all the standards were correct. We ensured that the standards were accurate and that the method was reliable using the above methods. Target mRNA sequences with ideal melt curves and sizes were identified using SYBR quantitative real-time polymerase chain reaction (qRT-PCR) and 2.0% agarose gel electrophoresis. The primer sequences are shown in Supplemental Figure 2. Each sample was measured in triplicate. Scatterplots represented the mean value of all measurements.

Immunohistochemical Procedure and Analyses on Human Samples

Immunohistochemistry (IHC) of eutopic tissue samples was used to identify differentially expressed proteins between the 26 endometriosis patients and 26 endometriosis-free patients. For SHH signaling activation, immunoreactive SHH was evaluated in the cytoplasmic compartments of the stromal cells. All tissues were fixed in 10% formaldehyde, embedded in paraffin and cut into 4-μm sections. Immunohistological staining was conducted by boiling the sections in 10 mM citric acid, pH 7.0. The slides were incubated with a poly-clonal rabbit antibody (1:200 dilution; Biosis) for 2 hours at 37°C. The sections were washed in phosphate buffer saline (PBS) three times and then incubated with mouse anti-rabbit secondary antibody for 40 minutes at 37°C. Peroxidase substrate containing 3, 3′-diaminobenzidine tetrahydrochloride chromogen was added to the sections for 2 minutes to develop the reaction. All slides were examined and scored by two independent pathologists who were blinded to both the clinical and pathological data. The quantification of the selected proteins was performed using Image-Pro Plus 6.0 (Media Cybernetics). Scoring was carried out for the mean density (ratio of integrated optical density SUM/area). Ovarian cancer tissues were used as positive controls, and the absence of the primary antibody served as the negative control.

Statistical Analyses

All statistical analyses were performed using the GraphPad Prism version 5.0 statistical software (GraphPad), R (version 3.1.3; R Core Team), and SAS (version 9.4; SAS Institute). The GraphPad Prism statistical software analysis and Adobe Illustrator CS5 (Adobe Systems) were used for figure generation. mRNA data are presented as the fold change relative to the endometriosis-free group. The differences of SHH protein levels considering the two groups (eutopic endometrium from endometriosis and controls) were evaluated using the t' Test. As the other clinical data were not normally distributed, all data were expressed as a median and interquartile range (IQR) and differences between the two groups were analyzed with the Wilcoxon test. Pearson analysis was carried out to identify correlations. *Receiver operating characteristic* (ROC) curves were generated to determine the diagnostic utility of the mRNAs using R Language. $P < .05$ was considered statistically significant.

RESULTS

Identification of Differentially Expressed mRNAs in the Tissue Samples of Endometriosis

Using qRT-PCR, we first measured the mRNA levels of the SHH pathway members (*SHH, Patched 1, SMO, GLI1, GLI2, GLI3, and SUFU*) in the eutopic endometria of the women with and without endometriosis. Four of the SHH pathway molecules were differentially expressed between the endometriosis samples and controls. For example, the endometriosis patients presented higher transcript levels of *SHH* (Fig. 1A, $P < .001$), *SMO* (Fig. 1B, $P < .05$), *GLI1* (Fig. 1C, $P < .01$), and *GLI3* (Fig. 1D, $P < .001$) than the controls, while no statistical significance was evident for the other members of this pathway. Thus, these differentially expressed mRNAs may function in the endometriosis eutopic endometrium.

Validation of Specific Molecules that were Differentially Expressed in the Tissue Samples

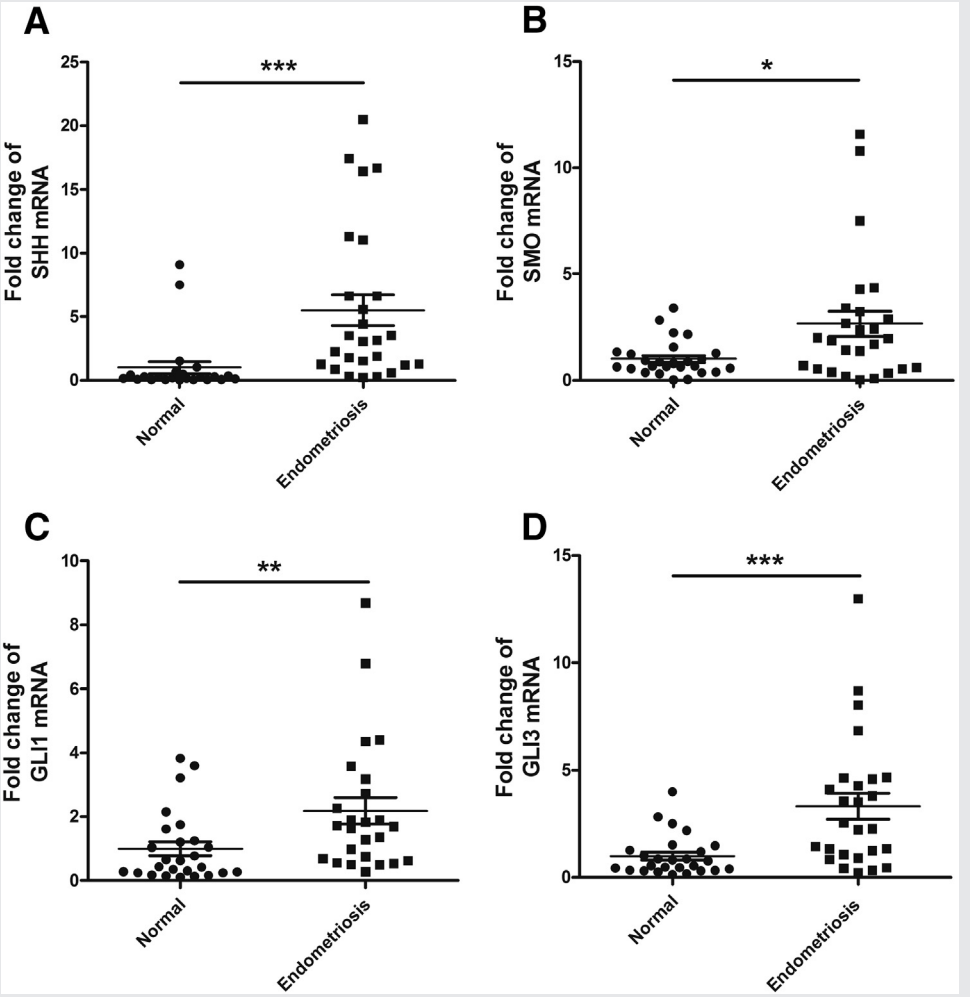
To further confirm whether these molecules were also abnormally expressed at the protein level, we performed an IHC analysis of the different tissue samples, namely, the eutopic endometria with and without endometriosis. The endometriosis eutopic tissues demonstrated positive staining for *SHH* (Fig. 2E), *SMO* (Fig. 2F), *GLI1* (Fig. 2G), and *GLI3* (Fig. 2H) in both the glandular and stromal nuclei and the cytoplasm compared to the control tissues (Fig. 2A–D). The immunostaining of SHH signaling component expression was analyzed by quantifying the IHC mean density. The results showed enhanced expression levels of *SHH* (Fig. 2I), *SMO* (Fig. 2J), *GLI1* (Fig. 2K), and *GLI3* (Fig. 2L) in the endometriosis samples compared to the other endometrial samples ($P < .001$).

In addition, the associations between SHH signaling molecule expression and the endometriosis score and preoperative values of CA125 for endometriosis are shown in Supplemental Figure 3. The SHH signaling molecules showed significant linear correlations with the endometriosis score and preoperative values of CA125 in this disease. These findings revealed an important association between the SHH signaling pathway and the clinical indicators of endometriosis.

Diagnostic Efficiency of SHH Signaling for Endometriosis

The results just mentioned showed that the endometriosis patients displayed a highly characteristic expression. We next endeavored to evaluate the diagnostic value of these aberrantly expressed genes for this disease. ROC curves were generated to evaluate the expression levels of the genes mentioned above, and the associated area under the ROC curve (AUC) and the sensitivity and specificity were used to confirm the diagnostic potencies of these genes. As shown in Figure 3, the highest AUC value was for *SHH*, which reached 88.13% (Fig. 3A), and the lowest AUC was for *SMO*, at 60.95% (Fig. 3B). In addition, ROC curve analysis showed that the AUCs of *GLI1* (Fig. 3C) and *GLI3* (Fig. 3D) were 75.74% and 78.85%, respectively. In the context of the ROC

FIGURE 1



Aberrant expression profile of mRNAs in eutopic lesions between endometriosis and controls. The expression levels of special mRNAs in the tissue samples: (**A**) *sonic hedgehog*, (**B**) *SMO*, (**C**) *GLI1*, and (**D**) *GLI3*. Data (median and interquartile range were expressed as the fold change and obtained from 26 eutopic lesion RNAs per experimental group, with each lesion isolated from an individual sample. The Wilcoxon test was used when the comparison was between different mRNA data. **P*<.05, ***P*<.01, ****P*<.001.

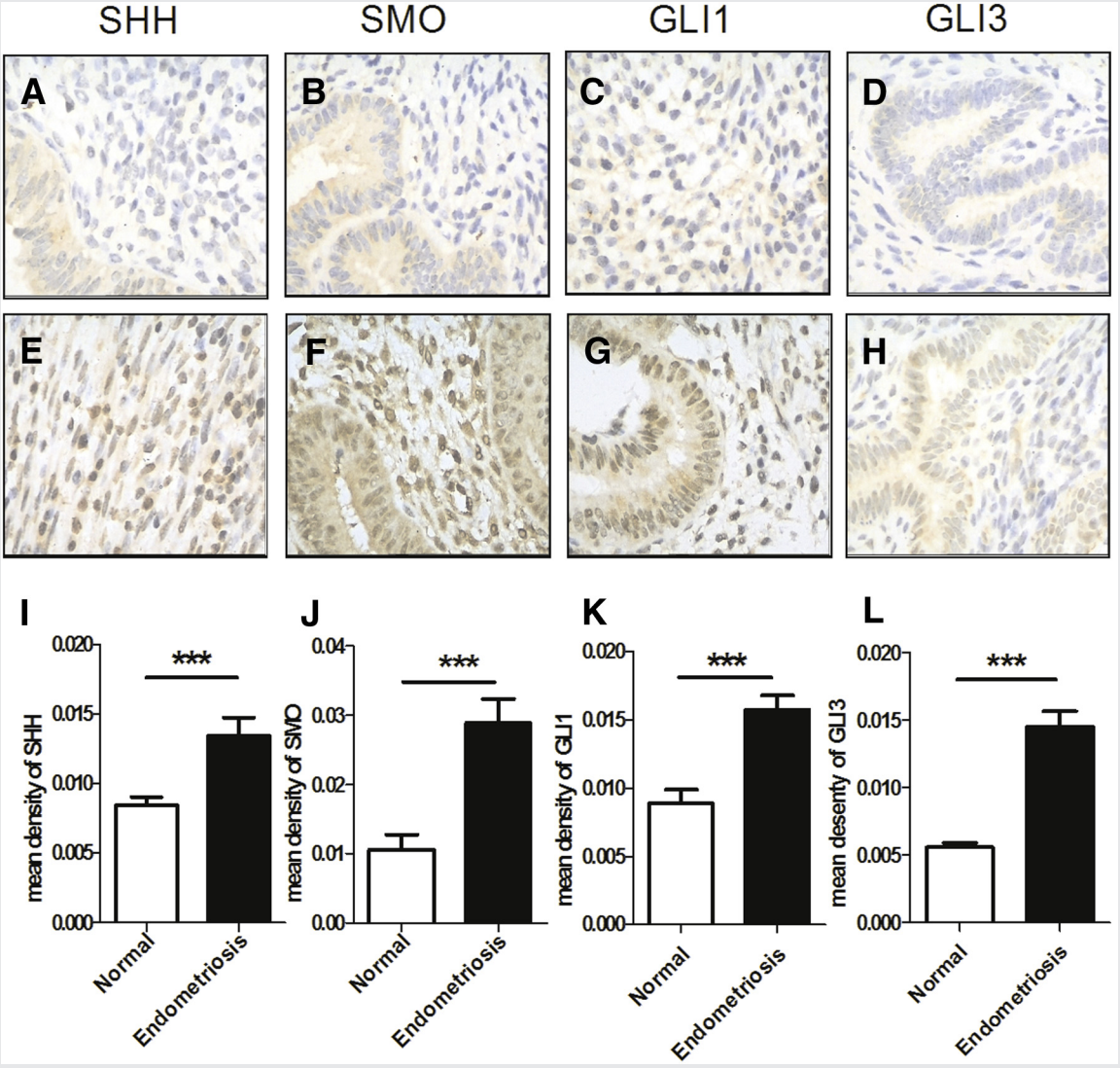
He. Activation of the SHH pathway in endometriosis. Fertil Steril 2018.

curve, an AUC value of 1.0 suggested that the SHH signaling pathway being tested was ranked at the top, whereas a value of 0.5 indicated that the SHH signaling being tested was randomly ranked within the list. Moreover, we found that *GLI1* had the greatest sensitivity, which was 96.15% at the cutoff point among the specific SHH pathway components (Fig. 3C), whereas *SMO* had the greatest specificity, which was 92.31% at the cutoff point (Fig. 3B). These results suggest that specific genes in the SHH signaling pathway are potential biomarkers for discriminating endometriosis.

Differentially Expressed SHH Pathway Genes were Associated with the Pathological

The differentially expressed SHH pathway was also analyzed to predict its functions in disease development. We examined whether the abnormally expressed SHH pathway could predict the clinical features of endometriosis, including pouch of Douglas obliteration involvement and the revised AFS score. This evaluation revealed that the expression levels of a set of molecules varied. For example, the *SHH, SMO, GLI1 and GLI3* levels were increased in the endometria from the patients with the pouch of Douglas obliteration caused by endometriosis (n=15) compared to the patients without the pouch of Douglas obliteration (n=11; Fig. 4A–D). ROC curve analysis showed that *SMO* presented the highest AUC of 0.8410 (95% confidence interval [CI] 0.6933–0.9887, $P<.05$) with 93.33% sensitivity and 61.54% specificity (Fig. 4B). Subsequently, a comparison of the revised AFS score indicated that *SHH, SMO, GLI1 and GLI3* showed significantly increased expression levels in advanced (III-IV) patients (Fig. 4E–H). *SMO* also had the highest AUC at 0.9479 (95% CI 0.8679–1, $P<.05$), with the best sensitivity and 81.25% specificity (Fig. 4F). Additionally, we correlated the

FIGURE 2



The expression of sonic hedgehog (SHH) signaling pathway molecules varies within endometriosis tissues. The immunostaining of tissue samples shows the protein expression levels of (**A**) *SHH*, (**B**) *SMO*, (**C**) *GLI1*, and (**D**) *GLI3* in the control group, and (**E**) *SHH*, (**F**) *SMO*, (**G**) *GLI1*, and (**H**) *GLI3* in the eutopic endometria of endometriosis group. The 26 eutopic lesions per experimental group were assessed by immunohistochemistry. To quantify the protein expression levels, ten random areas over each tissue were determined. The magnification was 400×. (**I-L**) Quantitative mean density. Differences between the clinical data were analyzed using a t' Test (*SHH*) and the Wilcoxon test (*SMO, GLI1, and GLI3*). ***$P<.001$.

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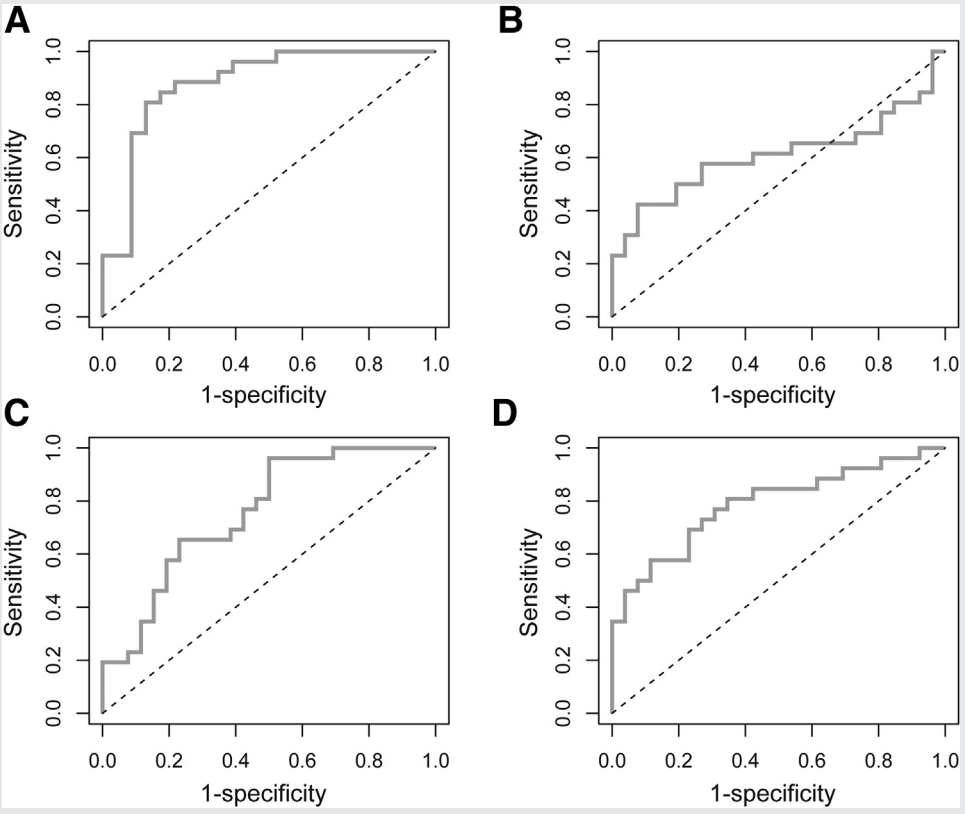
expression of these circulatory mRNAs with the phases of the menstrual cycle. However, no statistically significant differences in the expression levels were found between the proliferative and secretory phases in the endometriosis patients. Collectively, these results suggested that the four protective molecules (*SHH, SMO, GLI1, and GLI3*) could discriminate the severe versus mild stages of the disease and other associated clinical features.

DISSCUSION

Endometriosis is a common benign gynecological disorder that has many features of malignant disease, such as excessive proliferation, migration and invasion. Here, we demonstrate that a malignancy-related signaling pathway, the SHH signaling pathway, is overexpressed in the eutopic endometria of patients with endometriosis. This finding provides further evidence that elevated SHH signaling pathway expression in ectopic endometriotic lesions may be associated with endometriosis.

Endometriosis exhibits proliferation, implantation and angiogenesis, supporting an oncogenic role for Eu (21). Previous reports have indicated that abnormal activation of the SHH signaling pathway is associated with the advanced stages of breast cancer (22) and ovarian cancer (23), suggesting that the SHH signaling pathway may facilitate the disease

FIGURE 3



Assessment of the diagnostic accuracy of these specific molecules for endometriosis. *Receiver operating characteristic* curve analyses revealed that the tissue levels of (**A**) *sonic hedgehog*, (**B**) *SMO*, (**C**) *GLI1*, and (**D**) *GLI3* were useful biomarkers for differentiating the women with and without endometriosis, with area under the curve values of 97.14% (95% CI 0.9259–1), 66.07% (95% CI 0.4919–0.8296), 86.43% (95% CI 0.7224–1), and 88.33% (95% CI 0.7768–0.9899), respectively.

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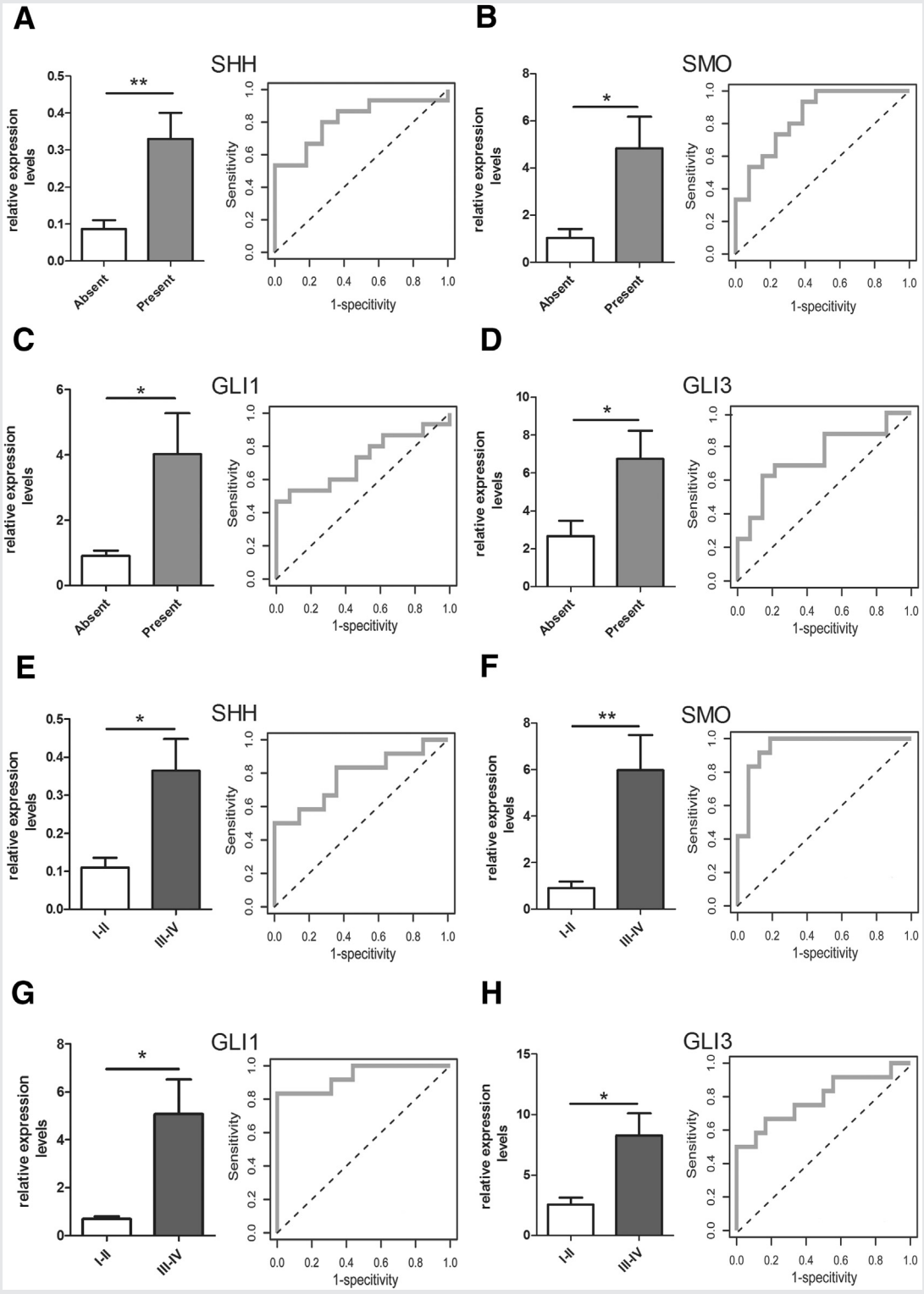
progression of these reproductive malignancies. In addition, endometriosis is associated with steroid signaling, especially estrogen and progesterone signaling (24). Elevated HH signaling expression correlates with a positive progesterone receptor status in human trophoblasts (25). In the current study, we demonstrated that SHH pathway activation occurred in a manner that was similar to that observed in tumors resembling human endometriosis found in a KLF9-deficient mouse model (13). In contrast, despite the differing steroid levels in the proliferative and secretory phases of the menstrual cycle, the expression of the SHH signaling pathway components was not altered during the menstrual cycle in the endometrial tissues derived from the women either with or without endometriosis.

Specifically, lower expression of *SMO* was associated with faster tumor progression and shorter disease-free survival after surgery than the controls. The distinct findings of these studies may represent the different functions of the SHH signaling pathway, namely, the promoting of migration and invasion, in different disease contexts. Moreover, in our study, strong nuclear staining of *GLI1* and *GLI3* was mainly observed in the Eu glandular epithelial cells, which are the same cells that express progenitor or stem cell markers (26). Coincidentally, evidence of the stem cell origin of Eu has emerged (27). Predictably, the overexpression of the SHH pathway components occurred in the endometrial samples in which the stem cells had high proliferative abilities in human endometriosis. These observations are consistent with the theory of a previous study that reported a 3-step process of pathogenesis (attachment, aggression, angiogenesis) in endometriosis (28).

One of the limitations of our study is the recruitment of women for the control group. Endometrial tissues from women without any known pathological condition would be more appropriate controls for our study. However, the restrictions by the research ethical board have kept us from obtaining endometrial biopsies from disease-free women as controls. Thus, endometriosis-free patients with idiopathic infertility served as controls in our study. Although we could not rule out the possibilities that these pathological conditions might have altered the expression of the SHH signaling pathway molecules in the endometrium, elevated *SHH, SMO, GLI1, and GLI3* expression were consistently found in endometriosis compared with the control endometria in our study. Furthermore, utilizing in vitro cell experiments to confirm the role of the SHH signaling pathway in the disease progression

FIGURE 4



Association between mRNA expression and the clinical features of endometriosis. The mRNAs with different expression levels in the eutopic endometria from patients with different clinical features, such as (**A–D**, *left*) with or without pouch of Douglas obliteration and (**E–H**, *left*) revised American Fertility Society score. (**A–H**, *right*) The diagnostic value of specific mRNAs for the pouch of Douglas obliteration and revised American Fertility Society score of endometriosis. Differences between clinical data were analyzed using the Wilcoxon test. **P*<.05, ***P*<.01.

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of endometriosis would also be a worthwhile investigation in our future studies.

In summary, our study has demonstrated that the expression levels of *SHH, SMO, GLI1, and GLI3* are elevated in patients with endometriosis compared patients without endometriosis. Functionally, our study presents promising diagnostic tools for endometriosis, suggesting that these molecules may play important roles in the diagnosis of endometriosis. Our findings are the first time to reveal the activation of the SHH signaling pathway in the eutopic endometrium of endometriosis, which improves our understanding of the pathogenesis of endometriosis. In addition, our findings have evaluated the potential of these molecules as promising diagnostic markers for this disease. Overall, this study offers new evidence toward understanding the etiology of endometriosis and diagnosing the disease.

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Activación anormal de la vía de señalización *sonic hedgeoh* en endometriosis y su capacidad diagnóstica

Objetivo: investigar la expresión anormal de las moléculas de señalización *sonic hedgehog* (SHH) en 52 tejidos endometriales eutópicos y su capacidad diagnóstica en endometriosis.

Diseño: estudio retrospectivo.

Lugar: hospital universitario.

Paciente (s): Se seleccionaron 26 mujeres con endometriosis histológicamente confirmada y 26 mujeres con endometrio histológicamente normal, la cuales fueron sometidas a un legrado o histerectomía.

Intervención (es): ninguna.

Medidas de los resultados principales: Los niveles de ARNm y de proteínas de las moléculas de la vía de señalización SHH.

Resultado (s): Los niveles de SHH, smoothened, dedo 3 de zinc de la familia GLI y su factor de transcripción (GLI1) en sentido descendente no solo fueron sobre-regulados en el endometrio eutópico de las pacientes con endometriosis comparado con el endometrio control sino también predijeron de forma independiente el inicio y la gravedad de la enfermedad.

Conclusión (es): Este es el primer estudio en revelar diferencias en cuanto a la activación de la vía de señalización SHH entre mujeres con y sin endometriosis y sugiere que la vía de señalización de SHH tiene capacidad de diagnosticar endometriosis.

SUPPLEMENTAL FIGURE 1

Characteristic	Endometriosis (n=26)	Control (n=26)
Age	36.4 ± 6.2	33.1 ± 2.9
Main Diagnose	Adenomyosis and Chocolate	Idiopathic infertility
Stage of the menstrual cycle		
Proliferation phase	18	20
Secretory phase	8	6
Pelvic adhesion	Caused by endometriosis	Caused by idiopathic infertility
President	21	20
Absent	5	6
r-AFS		
Stage I-II	14	NA[a]
Stage III-IV	12	NA
Serum CA125 level,mean±SD		
Stage I-II	70.6 ± 38.3	NA
Stage III-IV	106.6 ± 73.7	NA
Reopoerative causes		
Pelvic pain	8	NA
Dysmenorrhea	13	NA
Infertility	2	26
AUB[b]	3	NA
Distribution of Endometriosis		
Ovarian Endometrioma	16	NA
Adenomyosis	9	NA
Peritoneal Lesion	1	NA
DIE status		
With DIE[c] lesions	5	NA
Without DIE lesions	21	NA
VAS[d]		
2	42.9 ± 5.4	NA
2—5	51.7 ± 13.4	NA
6—9	56.3 ± 7.2	NA

Clinical characteristics of all samples used in the study. Values are presented as the mean ± standard deviation. [a]NA = not applicable; [b]AUB = abnormal uterine bleeding; [c]DIE = deep infiltrating endometriosis; [d]VAS = visual analogue scale.

He. Activation of the SHH pathway in endometriosis. Fertil Steril 2018.

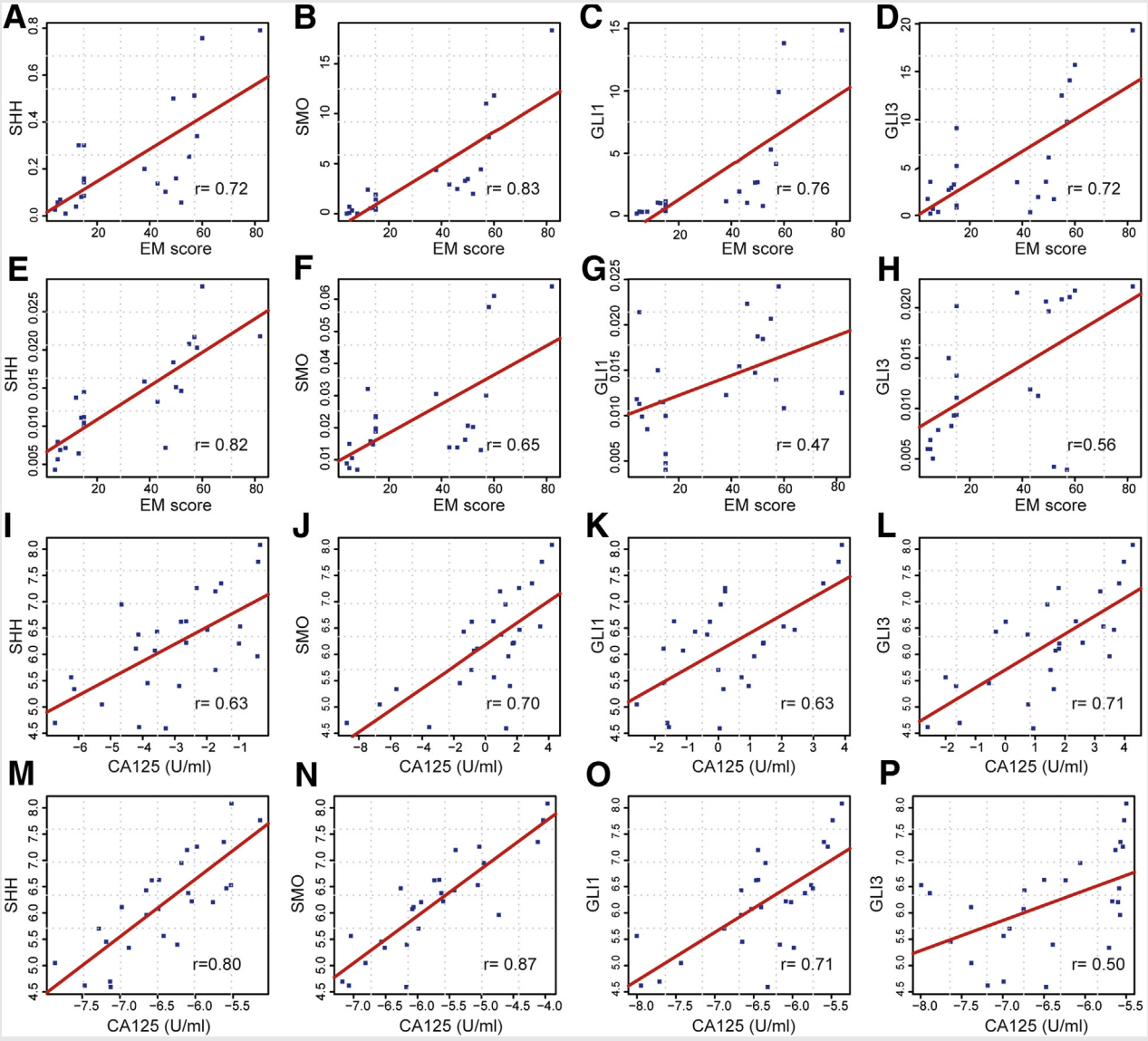
SUPPLEMENTAL FIGURE 2

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
SHH	5'-GGCAGGGGGTTCGGGAAGAGGAG-	5'-CGGGGTTGTAATTGGGGGTGAGT-3'
SMO	5'-TCACCTGGTCACTCCCCTTTGTC-3'	5'-CCGCACGGTATCGGTAGTTCTTG-3'
GLI1	5'-GCCTCGGGCACCATCCATTTCTA-3'	5'-ACTGTCTGTATTGGCTGCACTCC-3'
GLI3	5'-CCCAGGAATGGTTACATGGAGCC-3'	5'-CGGAAGTCATATGCAATGGAGGA-3'
GAP	5'-CAGGGCTGCTTTTAACTCTGGT-3'	5'-GATTTTGGAGGGATCTCGCT-3'

Sequences of the primers used for quantitative real-time polymerase chain reaction.

He. Activation of the SHH pathway in endometriosis. Fertil Steril 2018.

SUPPLEMENTAL FIGURE 3



Association between sonic hedgehog signaling molecule expression and Endometriosis (EM) score or the preoperative value of CA125 for EM. (A-H) Both the mRNA and protein levels of sonic hedgehog signaling molecules showed significant linear correlations with the EM score and (I-P) the preoperative values of CA125 for this disease. Correlation coefficients were determined with Pearson's correlation test.

He. Activation of the SHH pathway in endometriosis. Fertil Steril 2018.