ORIGINAL ARTICLES

The study of relationship between the expression of VEGF and Ang-2 and Tie-2 in endometriosis

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Abstract

Objective: To detect the expression of VEGF (vascular endothelial growth factor), Ang-2 (angiopoietin-2) and Tie-2 in endometriosis (EMs) and their correlations with menstrual cycle and clinical stage.
Methods: Thirty specimens of normal endometrium, eutopic endometrium and ectopic endometrium tissues were collected. The expression of VEGF, Ang-2 and Tie-2 was detected by immunohistochemistry methods.
Results: The positive rate of expression of VEGF in the normal endometrium, eutopic endometrium and ectopic endometrium tissues gradually increased (16.67%, 86.67%, 90.00%), compared three groups ($p < .01$). The positive rate of expression of Ang-2 in the normal endometrium, eutopic endometrium and ectopic endometrium tissues was 33.33%, 70.00%, 60.00%, respectively ($p < .05$). The positive rate of expression of Tie-2 in the normal endometrial, eutopic endometrium and ectopic endometrial tissues was 13.33%, 50.00%, 40.00%, respectively ($p < .01$). The expression of VEGF, Ang-2 and Tie-2 was closely correlated with each other ($r = 0.875$, $r = 0.905$, $r = 0.898$).
Conclusions: Our findings suggest that there is a close relation between VEGF, Ang-2 and Tie-2, the up-regulation of VEGF and Ang-2 may cooperatively contribute to survival and invasion of EMs. This may provide new targets for therapy of EMs.

Key Words: Vascular endothelial growth factor, Angiopoietin-2, Tie-2, Endometriosis, Immunohistochemistry

Endometriosis (EMs) is a common gynecological and frequently-occurring disease, seriously affecting the general health and quality of life of women. Among women of childbearing age, the incidence of the disease is 5% to 15%,[1] and the incidence of infertile women is up to 40% to 50%. At present, its exact pathogenesis still remains unclear. With the development of molecular biology and the further study of angiogenesis theory, more and more evidences indicate that angiogenesis is an important part of the development and progression of EMs. The angiogenic disorder of the endometrium may be the basis of the pathogenesis of EMs. The role of Ang-2 in EMs is not clear, and the correlation between the expression of Ang-2 (angiopoietin-2) and VEGF (vascular endothelial growth factor), in EMs has not been determined. There are no consistent conclusions at home and abroad. Therefore, immunohistochemical method was used to detect the expression of VEGF, Ang-2 and its receptor Tie-2 in EMs specimens. The relationship between the three factors and EMs was discussed for the first time. We hope to provide valuable laboratory data of the early diagnosis and prognosis of EMs, and provide a reliable theoretical basis for the development of new ways of effective clinical treatment.

1 Data and methods

1.1 General information

1.1.1 EMs group

A total of 30 specimens of EMs from February 2006 to September 2008 in the Third Affiliated Hospital of Inner Mongolia Medical College were identified by pathology.

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The age of the patients was 25-49 years old. Endometrial tissue was taken as the eutopic endometrium group. The ovarian follicle tissue was taken as the ectopic endometrium group. Tissues were fixed by 4% neutral formalin immediately, followed by conventional dehydration and paraffin embedding. After routine HE staining, histological morphology was observed under light microscope and tissue specimens were screened.

1.1.2 Normal control group

A total of 30 cases of normal endometrium of patients who had undergone hysterectomy or curettage and pathologically confirmed, were selected for the same period. The age in normal control group was about 24-48 years old.

All cases were not received hormone therapy six months prior to surgery, IUD was not placed. There was no significant difference between the two groups ($p > .05$). All selected specimens were confirmed by histopathology.

1.1.3 Experimental methods

Immunohistochemical S-P method was used for the study. Rabbit anti-human VEGF polyclonal antibody, rabbit anti-human Ang-2 polyclonal antibody and rabbit anti-human Tie-2 polyclonal antibody were purchased from Wuhan Boster Biological. DAB reagent and S-P kit were purchased from Wuhan Boster Biological Company. The experimental steps were carried out according to the instructions.

1.1.4 Determination of results

VEGF, Ang-2 and their receptor Tie-2 were determined by immunohistochemical S-P method. The brown yellow or brown particles appeared in the cytoplasm of the specimens were considered as positive after immunohistochemical staining. In the northern center of the image analysis of Cmais color pathological image system, semi-quantitative integral method proposed by Frank was used to classify the percentage of positive cells and positive staining intensity according to the score, and then determine the positive intensity on the basis of the two scores. Specific scoring criteria are as follows: (1) The percentage of positive cells is < 5%, 0 point; 5%-25%, 1 point; 26%-50%, 2 points; 51%-75%, 3 points; > 75%, 4 points. (2) The staining intensity of positive cells is colorless or coloring is weak, 0 point; light brown, 1 point; brown yellow, 2 points; dark brown or cytoplasm dark brown patches, 3 points. (1) + (2) is the total score of the organization. The total score is 0-7, 0 is negative, 1-3 points are weak positive (+), 4-7 points are strong positive (++-+++).

1.1.5 Statistical analysis

SPSS10.0 software was used for statistical analysis. The expression rates of VEGF, Ang-2 and Tie-2 were compared using χ^2 test or exact probability method; The correlation of VEGF, Ang-2 and Tie-2 was presented by spearman rank correlation analysis.

2 Results

2.1 The expression of VEGF, Ang-2 and Tie-2 in three groups

(1) In normal control group, the positive expression rate of VEGF was 16.67%. The positive expression rate of EMs in eutopic endometrium group was 86.67%. In the ectopic endometrium group, the positive rate was 90.00%. The χ^2 test showed that there was a significant difference in the positive rate of VEGF among the three groups ($p < .01$). The positive rate of EMs in normal endometrium, eutopic and ectopic endometrium showed significantly differences ($p < .01$), but there was no significant difference between eutopic and ectopic endometrium ($p > .01$).

(2) The positive expression rate of Ang-2 in normal control group was 33.33%. The positive rate of Ang-2 in EMs eutopic endometrium group was 70.00%. In ectopic endometrium group, the positive rate of Ang-2 was 60.00%. The positive rates of Ang-2 expression in the three groups were statistically different ($p < .05$) by χ^2 test. The positive rates of EMs in normal control group and eutopic endometrium group were significantly different ($p < .01$). However, the positive expression rates between eutopic and ectopic endometrium group, ectopic endometrium and normal control group had no statistical significance (all $p > .01$).

(3) The positive rate of Tie-2 in normal control group was 13.33%. The positive rate of Tie-2 in EMs eutopic endometrium group was 50%. And it was 40.00% in EMs ectopic endometrium group. The χ^2 test showed that there was a significant difference in the positive rate of Tie-2 among the three groups ($p < .01$). There was a significant difference between the normal control group and the eutopic endometrium group with regard to the positive expression rate of EMs ($p < .01$). However, the difference among the three groups was not significant (all $p > .01$, see Table 1).

2.2 Relationship between the expression of VEGF, Ang-2, Tie-2 in EMs

The relationship between the expression of VEGF, Ang-2 and Tie-2 in EMs was analyzed with the statistical software SPSS10.0. The results showed that VEGF was positively correlated with Ang-2 ($r = 0.875$, $p = .000$); VEGF was positively correlated with Tie-2 ($r = 0.898$, $p = .000$); Ang-2 was positively correlated with Tie-2 ($r = 0.905$, $p = .000$).

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Table 1: VEGF, Ang-2, Tie-2 expression in three groups

Group	Cases	VEGF (positive, %)	Ang-2 (positive, %)	Tie-2 (positive, %)
Control group	30	5 (16.67)	10 (33.33)	4 (13.33)
EMs eutopic endometrium group	30	26 (86.67)	21 (70.00)	15 (50.00)
EMs ectopic endometrium group	30	27 (90.00)	18 (60.00)	12 (40.00)

3 Discussion

VEGF is a multifunctional cytokine that acts specifically on vascular endothelial cells,[2] and has a strong role in promoting endothelial cell proliferation and blood vessel growth. It is recognized as the most critical pro-angiogenic factor at present. In 1993, for the first time, Shweiki et al.[3] found the rodent endometrial VEGF mRNA changes periodically with the menstrual cycle, and confirmed the expression of VEGF mRNA on both endometrial epithelial cells and stromal cells, which were regulated by estrogen and progestin, respectively. In 2002, Tan et al.[4] used RT-PCR method and found that the VEGF mRNA level in endometrium of patients with EMs was significantly higher than that of normal women. As a novel vascular growth factor family, the Ang family has become a hot spot of research in recent years. At present, the most recognized family members are Ang-1 and Ang-2. Ang-2 is a natural competitive antagonist of Ang-1 and its receptor is the endothelial-specific tyrosine kinase receptor (Tie-2), which can only be exerted when Ang-2 binds to Tie-2 on endothelial cells biological functions. Ang-2 is only expressed in the active site of revascularization in adult ovary, uterus, placenta and some tumor tissues. Geva et al.[5,6] showed that Ang-2 protein was expressed on the endometrium with normal menstrual cycle, suggesting that Ang-2 protein plays an important role in endometrial angiogenesis. In 2006, Hur et al.[7] found that the expression level of Ang-2 and protein in endometrium of EMs women was higher than that of controls, suggesting that the intimal EMs in female endometrium has higher angiogenesis activity, more invasive and prone to ectopic implantation. In recent years, it is proved that the adhesion, angiogenesis and invasive growth of ectopic endometrium are the basis for the formation of EMs. The angiogenesis is a complex process, which requires a variety of factors to interact with each other. The role of Ang-2 on blood vessels is closely related to the existence of other pro-angiogenic factors, especially VEGF. When VEGF is absent, Ang-2 expression mainly degenerates the blood vessels, reducing the number of blood vessels, accompanied by endothelial cell apoptosis. When Asahara et al. stimulated the corneal neovascularization in mice with Ang-1, Ang-2 and VEGF, they found that the effect of Ang-1 and Ang-2 alone could not promote angiogenesis. However, the synergistic effect of VEGF and Ang-2 promoted the increase of blood vessel length and the proliferation of capillary endothelial cells.

The experimental results showed that: (1) VEGF protein expression in endometrial specimens was mainly cytoplasm of glandular epithelial cells, and a small amount of interstitial cells were also expressed in cytoplasm. The expression of VEGF in eutopic and ectopic endometrium of EMs was higher than that in normal endometrium group, the difference was statistically significant. In 2000, Nisolle et al.[8] analyzed the ectopic lesions by immunohistochemistry. It was found that the VEGF staining of glandular epithelium was significantly higher than that of the normal endometrium. The experimental results are consistent with the findings of Nisolle. This study proved that VEGF and EMs were closely related. It is presumed that the increase of VEGF is associated with the specific receptor on the endothelial cells. Through the tyrosine kinase conduction pathway, it promotes the proliferation and angiogenesis of vascular endothelial cells. At the same time, it also increases the permeability of blood vessels, promotes the permeability of plasma protein, and induces the proliferation of endothelial cells, fibroblasts and inflammatory factors. Finally, a highly vascularized interstitial is formed, which provides a good premise for the development of EMs. (2) The expression of Ang-2 protein in endometrium was mainly in the cytoplasm of glandular epithelium, with a small amount of interstitial cells in the cytoplasm and vascular endothelium, but the expressions were weak. The expression of Tie-2 protein was dominated by glandular epithelial cytoplasm, and a small amount of vascular endothelial cells and cytoplasm of interstitial cells were expressed in varying degrees, but weak. This is consistent with the findings of Hirchenhain et al.[9] The experiment also showed that Ang-2 and Tie-2 protein expressions in EMs eutopic endometrium group were higher than that in the normal group. This is consistent with the findings of Hur et al.,[7] suggesting that endometrium may carry out stronger angiogenic activity. Such intima fragments can easily invade the peritoneal surface under the appropriate environment after passing through the bloodstream, promote the formation of neovascularization, and obtain sufficient blood supply to survive. (3) The expression of VEGF and Ang-2 in EMs tissues was closely related. It is speculated that the interaction between VEGF and Ang-2 promotes angiogenesis, which may lay a certain histological basis for the occurrence and development of EMs. Ang-2 is a natural competitive antagonist of Ang-1. When Ang-2 is combined with Tie-2 on endothelial cells, it can competitively block the effect of Ang-1, inhibit the interaction between supporting cells and weaken the stability of vascular structure, resulting in angiogenesis of capillaries and forming new blood vessels. In this experiment, the expression of Ang-2, VEGF and Tie-2 in EMs tissues was positively correlated. It is speculated that the specific binding of Ang-2 and Tie-2 after a series of effects may promote

the formation of new blood vessels under the synergistic effect of VEGF, and provide the premise for the growth of ectopic endometrium. Therefore, it is expected that the use of angiopoietin inhibitors can be used to treat EMs and avoid surgical treatment to relieve the pain of the patients. The clinical diagnosis of EMs, the severity and the effect of the treatment were determined by laboratory tests of the VEGF and Ang-2 levels in the eutopic endometrium. With the development of molecular biology, we are expected to block the expression of VEGF and Ang-2 from the gene level, which may be a new target for the treatment of EMs.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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