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XAV-939 INHIBITED ERα PROTEIN EXPRESSION AND CELL PROLIFERATION IN ADENOMYOSIS

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Abstract: Adenomyosis (ADM) is a benign gynecological disease, ERα can induce the occurrence and development of adenomyosis. XAV-939 is an inhibitor of Wnt/β-catenin signaling pathway. In order to study the effect of XAV-939 on ERα in adenomyosis, the disease model of adenomyosis was established, and the mice treatment with XAV-939 by intraperitoneal injection. The ERα expression and cell proliferation were detected through immunohistochemical assay. The results indicated that ERα expression and abnormal proliferation in adenomyosis mice were both inhibited by XAV-939.

Keywords: ERα, XAV-939, adenomyosis

1. Introduction

Adenomyosis is defined as the presence of endometrial glands and stroma infiltrated deep and haphazardly into the myometrium [1]. The clinical presentation of adenomyosis includes pelvic pain, abnormal uterine bleeding, and infertility [2-5]. It is a common gynecologic disorder with poorly understood pathogenesis and pathophysiology. ERα as a subtype of estrogen receptors (ER) can induce the development of adenomyosis. It is expressed in many gynecological tumor diseases. Breast cancer, endometrial cancer, endometriosis, adenomyosis and leiomyomas are diseases that grow in an estrogen-dependent fashion [6]. In adenomyosis, the overexpression of ER can induce the occurrence of EMT in adenomyosis, and promote the cell proliferation of adenomyosis thus promote the development of adenomyosis [7].

ERα also can mediate the regulation of a variety of signaling pathways, it has been found to mediate the regulation of Wnt/β-catenin signaling pathway [8-10]. Meanwhile, the Wnt/β-catenin pathway as a regulation pathway of proliferation and apoptosis was found to regulate the development of adenomyosis [11]. XAV-939 is an inhibitor of the Wnt/β-catenin pathway, can regulate Axin by inhibiting Tankyrase 1 and Tankyrase 2 [12], thus inhibit Wnt/β-catenin signaling pathway. So we wanted to investigate the effect of XAV-939 on the

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expression of ERα in adenomyosis mice, in this way may find a possible treatment medicine for adenomyosis.

2. Materials and Methods

2.1 Animals and experiment protocol

All animal experiments were performed according to the guidelines and animal procedures were approved by the Institutional Animal Care and Use Committee of Jinan University (Approval number: IACUC-20200905-01).

The experimental animal disease model program was carried out according to the protocol as reported previously [13]. The mice were housed in the cage under controlled condition (12 light/12 dark cycle at a constant temperature of ~ 23 ℃), food and water were available ad libitum. The female newborn mice were used to conduct experiments.

Twenty-four female neonatal ICR mice were randomly divided into two groups: TAM (tamoxifen) group and Control group. The TAM group was orally fed with 1mg/kg tamoxifen suspended in peanut oil/lecithin/condensed milk mixture (2:0.2:3, by volume) at a dose volume of 5 μl/g body weight from Day 2 to Day 5 after birth, while the Control group was fed with the same amount of solvent without tamoxifen. After the neonatal mice were given the drug, they were continued to feed until the Day 60 after birth. Three mice were randomly selected from the TAM group and Control group respectively and sacrificed to acquire the uteri for making the paraffin section. H&E staining was used to determine whether the adenomyosis disease model was successfully constructed.

After the model was successful, three mice were randomly selected from Control group and ADM group respectively for the next study. Then, the remaining mice were randomly divided into three groups: Control group (n=4), ADM group (n=4) and XAV-939 group (n=4). The XAV-939 group was intraperitoneal injection with 3mg/kg of XAV-939 (4%DMSO + corn oil + XAV-939) while the Control group and ADM group both intraperitoneal injection with the same dosage of solvent (4%DMSO + corn oil). All mice were injected for consecutive 7 days, and the samples were sacrificed one day after the completion of the injection.

2.2 H&E staining

The slices were baked for 1h at 60℃, then sections were deparaffinized in xylene, followed by 100%, 95%, 90% and 80% ethanol washes successively. After the slides were stained with

hematoxylin and eosin successively, the slices were sealed with neutral resins and photographed under the light microscope (Nikon, Tokyo).

2.3 Protein extraction and Western blotting

After obtaining the right of mice uteri, the homogenizer was used to tissue homogenate and the RIPA (Beyotime, China) lysis buffer (containing 10% PMSF) was added after ultrasound. After ice lysis for 30min, the lysate was centrifuged with 14000g for 15min at 4℃. The supernatant was taken to measure the concentration of proteins through the BCA detection kit (Beyotime, China). After electrophoresis and transferred to PVDF membrane (Millipore, USA), proteins were blocked with 5% skimmed milk (BD, USA) for 1h. Subsequently, the membrane was washed and incubated with ER (1:1000, Abcam) and anti-β-actin (1:1000, Cell Signaling) primary antibody overnight at 4℃. Afterwards, the membrane was incubated with HRP-conjugated goat-anti-rabbit IgG (1:8000, Cell Signaling) for 1h at room temperature. ECL chemiluminescence reagent (Millipore, USA) was used to determine the expression of protein according to the manufacturer's instructions.

2.4 Immunohistochemistry (IHC) analyses

The paraffin-embedded sections were deparaffinized and hydrated successively, antigen retrieval was performed with citrate buffer solution (pH6.0) for 15min, and endogenous peroxidase activity was eliminated by incubation in 3% hydrogen peroxide. Blocking was performed with 10% goat serum for 1h at room temperature, subsequently, the tissue sections were incubated with ERα (1:200, Santa Cruz), anti-PCNA (1:200, Proteintech) primary antibody overnight at 4℃. Then HRP-conjugated secondary antibody (1:200, Cell Signaling) was incubated with the tissues for 2h at room temperature. Immunoreactive proteins visualization were performed with the 3,3-N-Diaminobenzidine tetrahydrochloride (Sangon Biotech, China) and nuclei were counterstained with hematoxylin. The sections were observation under the light microscope (Nikon, Tokyo). Semiquantitative of immunoreactivity was determined by Image J (National Institutes of Health, USA), as described previously [14].

3. Statistical analysis

Statistical analyses were performed through GraphPad Prism 8 (GraphPad Software Inc., USA). Data were shown as the mean±SD. Unpaired two-tailed Student t test and One-way

ANOVA with Tukey's post-hoc test were used to evaluate the different of two groups and three groups respectively. P values <0.05 were considered significant.

4. Results

4.1 The adenomyosis disease model in mice is successful

The newborn mice were fed until Day 60, the uteri of the mice were obtained and H&E staining was used to diagnosis of adenomyosis. In figure 1, the myometrium of Control group was arrayed in bundles and well spaced from the endometrium, whereas in the TAM group, the endometrium gland was invaded into the muscular layer (black arrow). Meanwhile, the smooth muscle structure of the muscular layer was obviously disordered, and it's boundary with the endometrium was obscure. This result conformed with the definition of adenomyosis, it illustrated that the construction of adenomyosis was successful.

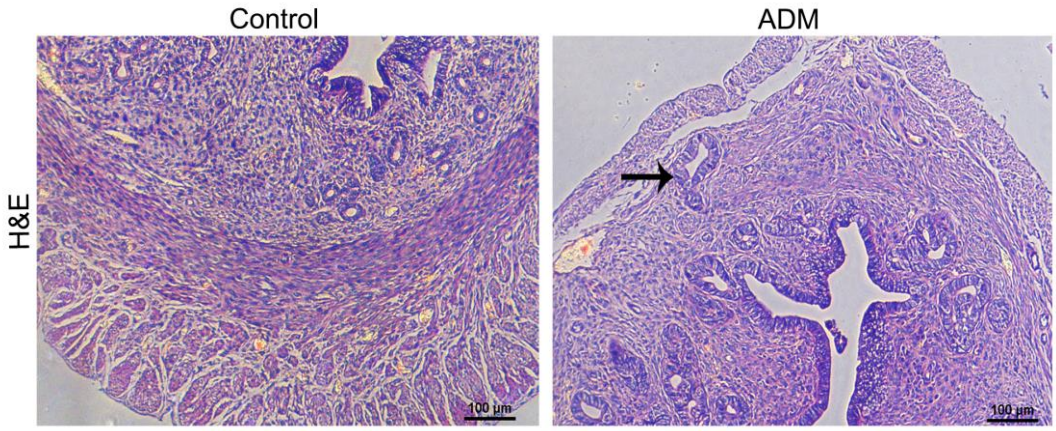

Figure 1. The H&E staining of uteri with mice.

4.2 ERα is upgraded in adenomyosis mice

Western blotting was performed to analyze the expression of ER. The level of ER protein in ADM group was higher than in Control group (Figure 2A). As a major subtype of ER, the IHC analysis revealed the expression of ERα protein in ADM group was raised versus the Control group (Figure 2B). The above indicated the ER and ERα proteins were increased in adenomyosis mice.

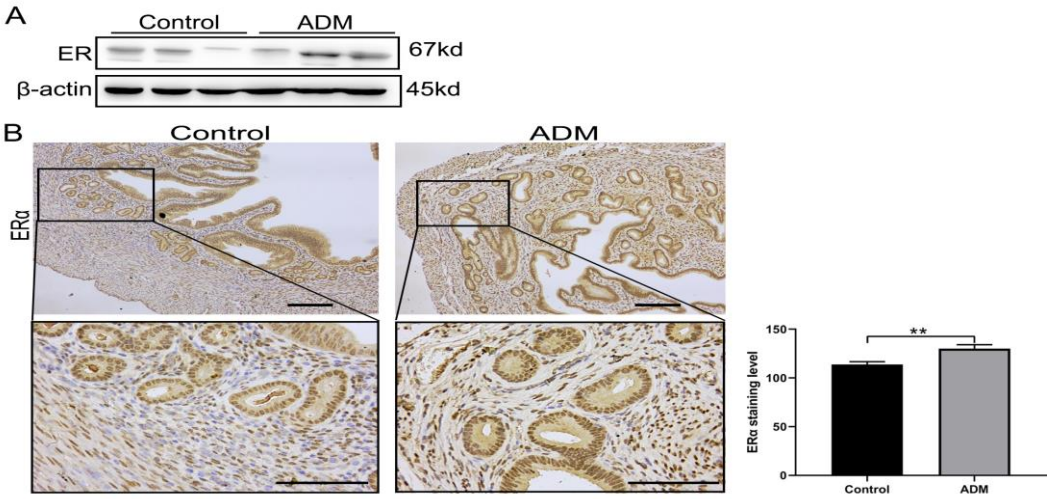

Figure 2. The expression of ER and ERα proteins in uteri with mice (**p<0.01).

4.3 The expression of ERα in adenomyosis mice is inhibited by XAV-939

In order to study the effect of Wnt pathway inhibitor XAV-939 to ERα, the mice were treated with XAV-939. The IHC analysis showed the expression of ERα in XAV-939 group was decreased compare to ADM group while ADM group was higher than Control group (Figure 3A), the data statistics was also illustrated the point (Figure 3B). The result illustrated the expression of ERα in adenomyosis mice was inhibited by XAV-939.

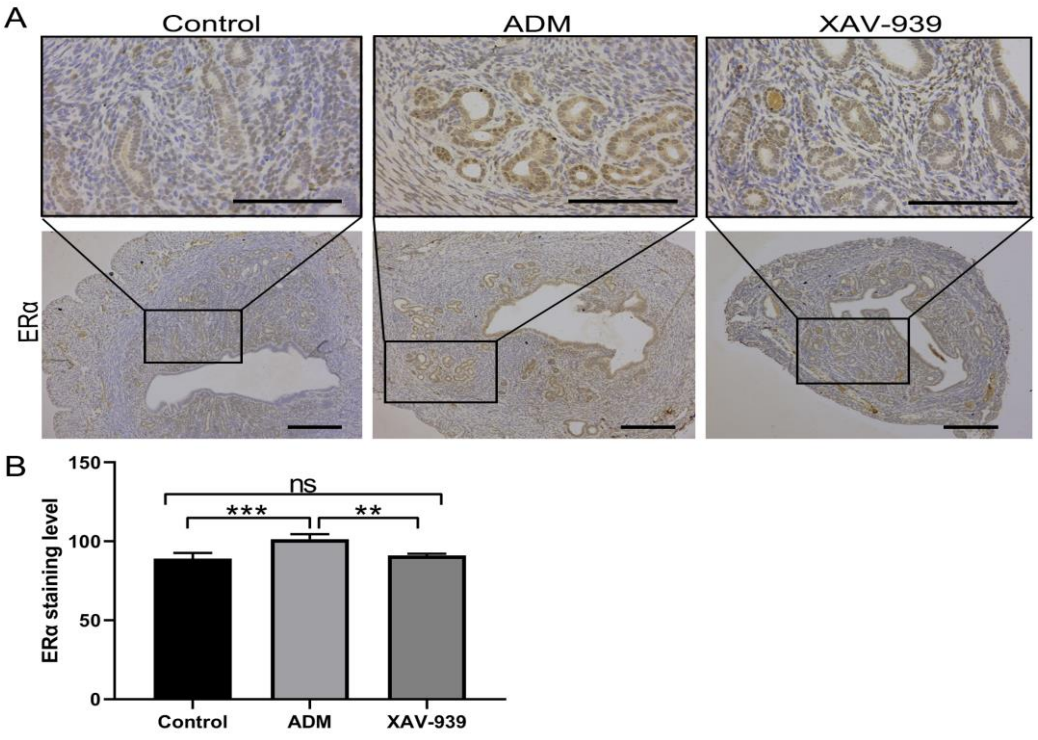

Figure 3. The expression of ERα in uteri of mice (**p<0.01, ***p<0.001).

4.4 The proliferation of adenomyosis mice is suppressed

PCNA was used to detected the proliferation situation of uterine tissues when the expression of ERα in adenomyosis mice was inhibited by XAV-939. The image showed the level of positive expression of PCNA in XAV-939 group was lower than ADM group while ADM group was higher than Control group (Figure 4). It indicated the proliferation of adenomyosis mice was suppressed when the expression of ERα in adenomyosis mice was inhibited by XAV-939.

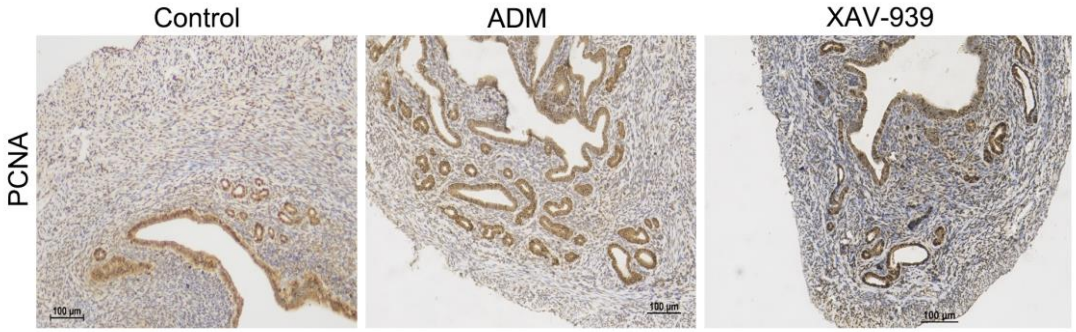

Figure 4. The PCNA expression in uteri of mice.

5. Conclusion

In summary, Wnt signal pathway inhibitor XAV-939 could inhibit the expression of ERα and may involve to the regulation of inhibiting to abnormal proliferation of adenomyosis. Namely, XAV-939 is contribute to ERα. The results providing a theoretical basis for the treatment of adenomyosis.

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