血管生成因子对怀孕子宫重造所扮演的角色

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計畫主持人：蔡美玲

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一、中文摘要

中文摘要

血管生長因子（vascular endothelial growth factor, VEGF）可促進細胞增殖、血管新生及血管舒張。在懷孕末期，一氧化氮調節的路徑會引起子宮的舒張。然而目前並無研究探VEGF是否與子宮重塑有關。因此，這研究是要探討在VEGF是否與內皮型一氧化氮合成脢的活化及子宮的重塑有關係。第一年我們的實驗以不同懷孕階段的大白鼠子宮作為動物模式，探討一氧化氮合成脢（nitric oxide synthase, eNOS）蛋白質表現的關係及一氧化氮產量的改變對內皮型一氧化氮合成脢表現的影響。依據實驗結果，懷孕第二十一天的子宮（G21）表現的內皮型一氧化氮合成脢表現量最低。與懷孕早期的子宮比較，懷孕末期的子宮表現內皮型一氧化氮合成脢表現量最高。於實驗室培養環境下，胚胎血管內皮型鈣離子依賴性黏著分子（VE-cadherin）蛋白質表現的關係及一氧化氮產量的改變對內皮型一氧化氮合成脢表現的影響。在一氧化氮抑制劑存在下，VEGF促進的血管內皮型鈣離子依賴性黏著分子表現可被抑制。

二、研究與目的

An expression of angiogenesis involves extracellular matrix degradation, endothelial cells migration, proliferation, cell-cell interaction and capillary formation. Vascular endothelial growth factor (VEGF), an angiogenic factor, is known to induce eNOS expression in human umbilical endothelial cells. NO pathway is known to cause uterine relaxation in late-gestation uteri. However, it is not clear whether the activation of eNOS is involved in uterine remodeling. Thus, my study was to examine whether the activation of eNOS by VEGF in uteri is associated with uterine remodeling. According to our purpose, pregnant uteri were used as our experimental model. Our first-year approach was to characterize the expression profile of eNOS during pregnancy. Our second-year approach was to characterize the expression profile of VE-cadherin during pregnancy and to examine the effects of VEGF on the expression of eNOS and VE-cadherin. Western blot analysis was used to measure the protein abundance of eNOS and VE-cadherin. Immunohistochemistry was used to localize eNOS and VE-cadherin.

Abstract

Vascular endothelial growth factor (VEGF) increases the production of nitric oxide (NO) by activating eNOS, which is involved in angiogenesis, vascular remodeling and vasorelaxation in human umbilical endothelial cells. NO pathway is known to cause uterine relaxation in late-gestation uteri. However, it is not clear whether the activation of eNOS is involved in uterine remodeling. Thus, my study was to examine whether the activation of eNOS by VEGF in uteri is associated with uterine remodeling. According to our purpose, pregnant uteri were used as our experimental model. Our first-year approach was to characterize the expression profile of eNOS during pregnancy. Our second-year approach was to characterize the expression profile of VE-cadherin during pregnancy and to examine the effects of VEGF on the expression of eNOS and VE-cadherin. Western blot analysis was used to measure the protein abundance of eNOS and VE-cadherin. Immunohistochemistry was used to localize eNOS and VE-cadherin.

Keywords: uterus, pregnancy, rats, eNOS.
maintenance of endothelial integrity, tube formation, and vascular remodeling (Vittet at al., 1997). The endothelium containing truncated VE-cadherin cannot undergo vascular remodeling (Carmeliet et al., 1999). Thus, VE-cadherin in endothelium is associated with vascular remodeling. In addition, VE-cadherin exists in the smooth muscles of an intact vessel. With the development of atherosclerosis, the distribution of VE-cadherin in the intact vessel has been altered (Bobryshev et al., 1999). As we know, implantation is an inflammation-like phenomenon. We speculate that VE-cadherin is present in pregnant uteri and its distribution may be associated with uterine development.

As mentioned previously, VE-cadherin is involved in vascular remodeling. Thus we hypothesized that the regulation of VE-cadherin expression by VEGF contributed to uterine remodeling in pregnant uteri. According to our purpose, three objectives were designed to 1) characterize the expression profile of VE-cadherin during pregnancy, 2) examine the effects of VEGF on the expression of eNOS and VE-cadherin, and 3) study the reversal effect of L-NAME on the expression of VE-cadherin.

三、材料與方法

Use and care of animals

Male (9-14 weeks) and female (8-12 weeks) Wistar rats weighing 200-350 g were housed in the colony at Animal Center of National Cheng Kung University Medical College at 24±1 ℃ under a 14-hr light (0500-1900) cycle. All experimental procedures and surgeries were performed in accordance with the guideline approved by the National Cheng Kung University Animal Care and Use Committee.

Animal models

Pregnant rats: Virgin female Wistar rats at pro-estrus, confirmed by virginal smear, were housed with a male rat. Full term of pregnancy for Wistar rats is about 22 days. Uteri from pregnant rats on gestation day 0 (G0), 7 (G7), 10 (G10), 14 (G14), 18 (G18) and 21 (G21) were used.

Human umbilical vein endothelial cells (HUVECs): To prepare the positive control for eNOS and VE-cadherin protein, HUVEC from Dr. Chauying J. Jen were used.

Uterine explant culture

The methods for uterine explants culture were adapted from the report of Tsai et al. (1997). Five strips, in a culture dish containing 3 ml of culture medium were cultured at 37 ℃ in a 5% CO₂ incubator for 24 h. The culture medium contained RPMI-1640 medium, 3µl of gentamicin (50 µM/ml, Sigma, MO), 1% of normal male rat serum, and various treatments. To examine the effects of NO modulators on the expression of eNOS, VEGF (Upstate Biotechnology, NY), L-N⁵-nitroarginine methyl ester (L-NAME, Sigma, MO) and L-Arginine (Sigma, MO) were used.

Preparation of homogenates for protein extraction

The tissue homogenate preparation was described previously (Tsai, et al., 2000). Tissue samples taken from G0, G7, G10, G14, 18 and G21 rats were minced in lysis buffer. After centrifuged at 7,500 g, 4 ℃ for 20 min by a microcentrifuge (Microfuge R, Beckman, CA), the pellet was discarded. Protein content in the supernatant was measured by the method of Lowry assay (Lowry, et al., 1958). Bovine serum albumin was used as the standard.

Immunoblot analysis of eNOS and VE-cadherin protein abundance

100 µg protein of tissue homogenate were loaded to each lane on 7.5 % SDS gel polyacrylamide gel. Protein molecular weight markers (NEL311, NEN, MA) was simultaneously added in one lane. The positive control from HUVEC was added in another lane. After electrophoresis was conducted. The gel was placed onto polyvinylidifluoride membrane (NEN, MA) for transferring at 4 ℃ overnight and was probed with primary antibody against eNOS and VE-cadherin Chemiluminescence reagent was added to the membrane according to the manufacturer protocol (ECL, NEN, MA). Finally, the membrane was exposed to the X-ray film (Kodak) for visualizing bands. A background control was obtained when the primary antibodies were not added. Autoradiograms were measured by a computerized image analyzer (PDI, Inc., NY).

Data analysis and statistical evaluation

All data were expressed as means ± SEM (standard errors of the mean). The data were analyzed by one-way analyses of variance (ANOVA) followed by LSD test in the Means model of SYSTAT. In all cases a p value less than 0.05 was considered statistically significant.

四、結果

The VE-cadherin was detected in pregnant uteri. When the protein abundance of eNOS in G0 uteri was expressed as 100 %, G7 was 96.5 ± 8.5 %, G10 was 90.4 ± 15.0 %, G14 was 43.1 ± 16.8 %, G18 was 54.9 ± 14.8 % and G21 was 27.5 ± 13.6 %. Relative to G21 uteri, G0, G7 and G10 uteri contained significantly greater amount of eNOS protein (p<0.05) The expression of VE-cadherin was significantly decreased in G14, G18 and G21 uteri. VEGF increased the expression of eNOS and VE-cadherin at lower concentrations. In the presence of VEGF, the increased VE-cadherin was reduced by L-NAME.

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Decidual development is initiated after the embryo attaches to endometrium. Penetration of trophoblast to the uterine epithelium and basement membrane leads to stromal proliferation and differentiation (Schlafke and Enders, 1975). Subsequently trophoblasts are differentiated to syncytiotrophoblasts and cytotrophoblasts, which then leads to the increase in vascular permeability and edema formation in stroma. The process of decidual development was observed in this study (Fig 2e, 3e and 4e).

Morphological changes in antimesometrium are different from that in mesometrium. Decidual development in both anti-mesometrium and mesometrium reach the maximal level on G10. Afterward, decidual cells undergo degeneration. In comparison, the degeneration of decidual cells in anti-mesometrium is much faster than that in mesometrium. In anti-mesometrium, most decidual cells have been degraded on G14 and completely disappeared on G18 and G21. In G18 and G21 uteri, there were only undifferentiated stroma cells between lumen epithelial cells and muscular layers. No decidual cells were observed. These results suggest that wall thinning in late gestation uterus is associated with decidual cells loss.

It is well accepted that implantation causes progressive changes in local environment. When mononuclear and multinuclear trophoblast interacts with maternal compartments, decidual tissues are formed and smooth muscles undergo proliferation and differentiation (Aplin et al., 1989; Glasser et al., 1991). Correspondent to cellular changes in pregnant uteri, syncytiotrophoblast and cytotrophoblast form villi. eNOS immunostaining can be detected in trophoblastic cells (Ariel et al., 1998). NO released by trophoblastic cells may play a role during the process of placentation (Ariel et al., 1998). Because VEGF induced VE-cadherin expression through a NO-dependent pathway, our data support that eNOS may play a role in decidualization during uterine remodeling.

NOS and VE-cadherin are presented in endothelial cells and associated with angiogenesis. Lung and placenta contain large amount of capillary structure. Our preliminary studies demonstrated that eNOS and VE-cadherin were expressed in lung, placenta, and G18 uterus. However, the expressions of eNOS and VE-cadherin in G18 uteri were more abundant than those in lung and placenta. This is the first study to demonstrate the presence of VE-cadherin in pregnant uteri. Because of different distribution, I speculate that eNOS and VE-cadherin may have other functions in uteri than angiogenesis.


