VEGF Expression and its Regulation by p53 Gene Transfection in Endometrial Carcinoma Cells

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Abstract: Vascular endothelial growth factor (VEGF) that activates endothelial cell growth induces angiogenesis, which is indispensable to tumorigenesis and tumor progression. On the other hand, tumor suppressor gene p53 has been considered to regulate VEGF expression, but the detailed relationship between them remains unclear. In this study, we aimed to study VEGF expression in endometrial carcinoma cells and the effect of p53 gene transfection on VEGF expression using p53-mutated endometrial carcinoma cell line, HEC-50B. Immunoblotting for detecting VEGF protein, p53 protein and β-actin was performed using 11 endometrial carcinoma cell lines. Levels of VEGF in the cultured media were measured by Enzyme Immunoassay (EIA). Transfection of wild p53 gene was carried out by SuperFect method in HEC-50B cells, which had mutant p53 gene and did not express p53 protein. The results of immunoblotting were analyzed by NIH image and expressed as values. The results of EIA were expressed as the relative value. The VEGF value was 0.8 ± 0.3 (n=6) in p53-wild group, whereas in p53-mutant group it was 1.6 ± 0.8 (n=5). VEGF expression was correlated significantly with p53 status (P<0.05). VEGF levels in p53 gene-transfected cells and the conditioned medium were decreased in 48 hours after p53 gene transfection. VEGF expression was down-regulated by p53 in endometrial carcinoma cells.

Key words: endometrial cancer, VEGF, p53, gene transfection, HEC-50

INTRODUCTION

Angiogenesis that is the formation of new capillaries from the pre-existing vascular network has an important role for wound healing in normal adult tissues. Especially in the female genital tract, it is correlated deeply with proliferation and differentiation of the endometrium, and provides a richly vascularized, receptive endometrium for implantation and placentation. Furthermore, it is inevitably necessary for a developing tumor to induce neovascularization around the tumor itself and receive the supply of nourishment and oxygen, especially when it grows up greater than 2 mm in diameter. Angiogenesis is of crucial importance for developing the metastasis of tumor cells as well as tumor growth itself, because tumor cells shed from the primary tumor site to distant body sites through blood vessels.
Vascular Endothelial Growth Factor (VEGF) and p53

Recently, many positive and negative angiogenic factors have been reported. Among them, VEGF was initially recognized as a vascular permeability factor, which induced ascitic fluid in tumor patients. It was then identified that VEGF activated vascular endothelial cell growth and promoted angiogenesis as well as increasing blood vessel permeability. VEGF is also expressed in the reproductive organs with rapid vascular endothelial turnover such as the endometrium and the placenta.

VEGF expression has been confirmed at various sites of carcinomas such as breast, lung, gastric, and colon cancers. VEGF gene expression in some of these cancers is reported that is regulated by hypoxia, cytokines, growth factors or tumor suppressor genes such as p53.

In an immunohistochemical study, the correlation between VEGF expression and a tumor suppressor gene p53, of which mutation was suggested to be implicated in tumorigenesis and tumor progression, has been examined recently. Some studies showed that high aberrant p53 expression was correlated with high VEGF expression. Yuan et al. reported that VEGF mRNA expression in tumors with high aberrant p53 expression was significantly higher than that in tumors with low aberrant p53 expression. However, there are contradictory findings in endometrial carcinoma. Recently, we described VEGF expression was significantly correlated with p53 expression by an immunohistochesmical study in endometrial carcinoma. Therefore, it is suggested that p53 may contribute to the regulation of VEGF expression.

To clarify the regulatory mechanism of VEGF expression by p53 in endometrial carcinoma, we examined both VEGF and p53 expressions in eleven endometrial carcinoma cell lines and those in the p53-mutated endometrial carcinoma cells after transfecting wild type p53 gene.

VEGF expression in endometrial carcinoma

1) Materials and Methods

Eleven endometrial cancer cell lines, including Ishikawa and HEC-1-A, -50B, -59, -88, -108, -116, -151, -251 and -265 (unpublished results) were used for detecting VEGF, p53 and β-actin expression. HEC-1-A, -50B, -59, -116 and -251 were defined to have the mutant p53 gene by polymerase chain reaction-single-strand conformation polymorphism and direct sequence analysis in our laboratory and other cell lines were defined to have wild p53 gene (unpublished results).

Endometrial carcinoma cells were harvested by dispersing the cells with 0.1% trypsin and 0.02% EDTA solution, and centrifuged three times with cold PBS. Then, the cells were lysed in Laemmli's sample buffer using SONIFIER 150 (Branson Ultrasunics Corporation, Danbury, U.S.A.). Ten microliters (2 μg/μl) of the lysates were heated at 100°C for 3 minutes, and loaded onto 12.5% sodium lauryl sulfate-polyacrylamide gel (e-PAGE1, ATTO, Tokyo, Japan) for the detection of VEGF and β-actin and onto 10% e-PAGE for p53. Subsequently, they were transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore, Tokyo, Japan). The membrane was incubated with Block Ace (Dainihon Seiyaku, Japan) and then either with anti-p53 antibody (DO-7, 1:1,000 dilution; Novocastra, Newcastle, United Kingdom), or with anti-VEGF antibody (A-20, 1:200 dilution; Santa Cruz, California, U.S.A.) in addition to anti-β-actin antibody (AC-15, 1:20,000 dilution; Sigma-Aldrich, Saint Louis, U.S.A.) for 1 h, and washed with PBS containing 0.3% Tween 20. This was followed by incubation either with horseradish peroxidase-linked rabbit IgG, (1:1,000 dilution; DAKO) or with horseradish peroxidase-linked mouse IgG, (NA931, 1:2,000 dilution; Amersham Pharmacia Biotech, Tokyo, Japan) for 1 h for p53 and β-actin, and washed with PBS containing 0.3% Tween 20. VEGF and β-actin was doubly stained. Bands were detected being exposed on an X-ray film by enhanced chemiluminescence technique (Amersham Pharmacia Biotech, Tokyo, Japan.). The VEGF and p53 protein was measured by densitometry using NIH (National Institutes of Health) Image software ver.1.62, and the amount was expressed as a relative value based on the amount of Ishikawa cells in the same membrane, which had been shown to have wild type p53 gene. One value corresponded to the VEGF and