The Role of Vascular Endothelial Growth Factor (VEGF) and p53 Status for Angiogenesis in Gastric Cancer

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Background: Angiogenesis is of crucial importance for tumor growth and development of metastases. Vascular endothelial growth factor (VEGF) has a potent angiogenic activity and mutations of the p53 gene has been thought to upregulate VEGF. The purpose of our study was to evaluate the prognostic significance of these tumor biomarkers for angiogenesis relative to the information derived from established clinicopathological parameters in gastric cancer.

Methods: In this study, we conducted an immunohistochemical investigation of VEGF and p53 expression in 145 tissue samples obtained from gastric cancer patients undergoing curative surgical treatment. To evaluate angiogenesis, microvessel density (MVD) was counted by staining endothelial cells immunohistochemically using anti-CD34 monoclonal antibody.

Results: High MVD was significantly associated with depth of tumor invasion and distant metastasis (p=0.004, 0.021, respectively). Moreover, overall survival for patients with high MVD were significantly lower than that of low MVD (p=0.048). Positive expression of VEGF correlated significantly with lymph node and distant metastasis (p=0.040, 0.048, respectively). However, no significant correlation was found between p53 expression and various clinicopathological parameters. VEGF positive tumors showed a higher MVD than VEGF negative tumors (p=0.028). The expression of p53 did not correlate with VEGF expression. Also, the relationship between the status of p53 expression and MVD had not statistically significant differences. In the multivariate analysis, status of VEGF, p53 expression and MVD were not an independent prognostic factor.

Conclusion: VEGF seems to be an important, clinically relevant inducer of angiogenesis and angiogenesis assessed by the MVD may be a useful marker for predicting metastasis in gastric cancer. However, further studies are warranted to clarify the impact of p53 on the angiogenesis and the prognostic significance of angiogenesis in gastric cancer.

Key Words: Angiogenesis; Genes, p53; Stomach Neoplasms; Immunohistochemistry

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INTRODUCTION

Angiogenesis has been shown to be a critical aspect of tumor growth and metastasis. The induction of angiogenesis by a tumor is controlled process, influenced by angiogenic and angiostatic factors which involves a complex interaction between tumor and endothelial cells. Among the many reported angiogenic factors, vascular endothelial growth factor (VEGF) is the most powerful endothelial-cell-specific mitogen that plays a key role in the complicated process of angiogenesis. It has been shown to be significantly upregulated in various human malignant tumors and to be associated with tumor angiogenesis and disease outcome.

Tumor growth and metastasis are characterized by uncontrolled cellular proliferation. This is usually the result of multiple genetic and epigenetic insults to the cell, particularly involving proto-oncogenes and tumor suppressor genes. The genetic and epigenetic alterations that are responsible for tumor growth and metastasis may underlie the ability of tumors to switch to an angiogenic phenotype.

p53 which encodes the tumor suppressor gene is mutated or deleted in about 50% of spontaneously arising tumors. Several studies have indicated that angiogenesis may be regulated, in part, by the function of the p53 tumor suppressor gene. Functional p53 suppresses angiogenesis by downregulating angiogenic factor expression, whereas dysfunctional p53 stimulates angiogenesis by both upregulating VEGF and downregulating thrombospondin-1, an angiogenesis inhibitor.

The degree of intratumoral microvessel density (MVD) is thought to reflect the angiogenic activity generated by the neoplastic cells and the supporting stroma. Moreover, tumor angiogenesis, as quantified by measurement of intratumoral MVD, has shown to be a significant negative prognostic factor in various human tumors, including breast carcinoma, lung carcinoma, prostate carcinoma, endometrial carcinoma, colon carcinoma, and gastric carcinoma.

The purpose of our study was to evaluate the prognostic significance of these tumor biomarkers for angiogenesis relative to the information derived from established clinicopathological parameters in gastric cancer.

MATERIALS AND METHODS

Patients and tumor specimens

The study included 145 patients who underwent curative surgery for gastric cancer at Chonnam National University Hospital between January 1992 and December 1993. Formalin-fixed and paraffin-embedded tissue blocks were selected by viewing original pathologic slides and choosing blocks that show the junction between carcinoma and benign tissue. This allowed for direct comparison of carcinoma and benign tissue side by side after immunohistochemistry. Patient characteristics, including sex, age, histologic grade, stage and survival data, were obtained by medical records and pathologist and physician contact when necessary. No patient had received anticancer therapy prior to the operation. The histologic grade was classified according to the criteria of Lauren and the World Health Organization. The tumors were staged at the time of surgery by the standard criteria for TNM staging using the American Joint Committee on Cancer. This study group comprised 99 males and 46 females. The mean age was 59.2±10.3 (mean±standard deviation) with a range from 28 to 79 years. The mean size of the tumor was 5.1±2.8 cm (mean±standard deviation) with a range from 0.5 to 15.0 cm.

Immunohistochemistry

All procedures for immunohistochemical staining were done by the Micro-Probe staining system (Fisher Scientific, Pittsburgh, PA) based on capillary action. Paraffin sections, of 4 µm in thickness with mounted probe on slides, were immunostained with anti-mouse monoclonal antibodies by the avidin-biotin peroxidase complex method.

Sections were deparaffinized and rehydrated. They were immersed in 0.6% hydrogen peroxide for 5 minutes to block the endogenous peroxidase activity. A polyclonal antibody against VEGF (A-20; diluted 1:50; Santa Cruz Biotechnology, Santa Cruz, Calif, USA), a monoclonal antibody against CD34 (QB-END/10; diluted 1:25; Novostra Lab., Newcastle, UK) and a monoclonal mouse antihuman p53 antibody (DO-7, diluted 1:100; Dakopatts, Glostrup, Denmark) were used as primary antibodies. The primary antibodies, in the aforementioned concentrations, were diluted in phosphate-buffered saline supplemented with 5% normal horse serum and 1% bovine serum albumin and then incubated with tissues for 15 minutes at 45°C. Anti-mouse immunoglobulin G (Sigma, St. Louis, MO) labeled with biotin was added as a secondary antibody for the detection of primary antibodies and the samples were incubated for 7 minutes at 45°C. After multiple rinses with universal buffer, streptavidin-alkaline phosphatase detection system (Biomed, Foster, CA) was applied for 7 minutes. As the final step, the slides were developed for 7 minutes with the enzyme substrate 3 amino-9-ethyl...