TGFβ-1 Regulation of VEGF Production by Breast Cancer Cells

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Background: Angiogenesis is essential for tumor growth and metastasis. Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor identified to date. TGFβ-1 acts as an indirect angiogenic agent.

Methods: VEGF and TGFβ-1 were measured in the serum of breast cancer patients and age-matched controls and in tumor tissue of cancer patients by ELISA. VEGF protein and mRNA expression by breast tumor cell lines were examined, and the effect of TGFβ-1 on VEGF production in these cells was assessed.

Results: VEGF levels were significantly higher (P = .03) in the serum of patients with breast cancer compared to age-matched controls. A positive correlation was found between serum (r = 0.539) and tumor tissue (r = 0.688) levels of VEGF and TGFβ-1. Metastatic MDA-MB-231 breast cancer cells produce more VEGF than do the primary BT474 cells. TGFβ-1 significantly (P < .05) increased production of VEGF.

Conclusions: Breast cancer cells constitutively produce VEGF protein and mRNA. There is a relationship between VEGF and TGFβ-1 levels in breast cancer patients, and TGFβ-1 regulates VEGF expression by breast cancer cells.

Key Words: Vascular endothelial growth factor—Transforming growth factor β-1—Breast cancer—Angiogenesis—Metastasis.
cell types, including tumor cells (15), vascular smooth muscle cells (16), and endothelial cells themselves (17). However, it is not the only regulator of VEGF, and factors such as insulin-like growth factor 1 (IGF-1) (18); interleukin 6 (IL-6) (19); wild-type, but not mutant, p53 (20); estradiol (21); and transforming growth factor β-1 (TGFβ-1) (22) may also play a role in the regulation of VEGF. Unlike many other angiogenic factors, TGFβ-1 does not induce endothelial cell proliferation but is capable of initiating angiogenesis indirectly in vivo (23). TGFβ-1-transfected CHO cells have been shown to grow more rapidly than parental cells and showed prominent angiogenesis when implanted into nude mice (24). Both of these effects were blocked by the use of anti-TGFβ-1 antibodies (24). This indirect angiogenic activity of TGFβ-1 may be accomplished by the induction of other angiogenic factors such as VEGF. TGFβ-1 has been shown to induce VEGF mRNA in mouse embryo fibroblasts and in a human lung adenocarcinoma cell line (22) and, more recently, VEGF protein in human glioma cell lines (25).

In this study we examined the role of VEGF in breast cancer. We hypothesized that VEGF is elevated in the serum of breast cancer patients and that this elevation may result, at least in part, from high levels of TGFβ-1 in both the serum and tumor tissue. Furthermore, we hypothesized that TGFβ-1 directly increases VEGF production by breast cancer cells. We therefore assayed VEGF and TGFβ-1 in both the serum and tumor tissue of breast cancer patients. VEGF expression in response to TGFβ-1 was assessed in primary and metastatic breast cancer cell lines.

METHODS

Serum Levels

Preoperative blood was collected from patients undergoing surgery for malignant breast disease (n = 26). No patients with a recent history of surgery or chemotherapy were included. Malignancy was histologically confirmed, and the series comprised of 21 ductal and 5 lobular carcinomas. Breast tumors were staged using the TNM staging system (26). Three patients were Tis, and the remaining patients were staged as follows: stage I, n = 8; stage II, n = 11; stage III, n = 3; and stage IV, n = 1. Samples were also collected from a group of healthy age-matched controls with no history of breast or other cancers. Blood was centrifuged at 11,000 x g for 5 minutes and the cleared homogenate collected and assayed for TGFβ-1 and VEGF by ELISA. The level of association between serum VEGF and TGFβ-1 was estimated by correlation, using the Pearson Product Moment Correlation coefficient.

Tumor Samples

Samples of tumor tissue were flash frozen in liquid nitrogen within 1 hour of resection and stored at −80°C. Specimens were diced and homogenized in a hand-held homogenizer in 500 μl of PBS containing 1 mM phenylmethylsulfonyl fluoride (PMSF). Samples were maintained at 4°C during the homogenization procedure. Debris was removed by centrifugation at 12,000 x g for 5 minutes and the cleared homogenate collected and assayed for TGFβ-1 and VEGF by ELISA. The level of association between tumor tissue VEGF and TGFβ-1 was estimated by correlation, using the Pearson Product Moment Correlation coefficient.

Cell Lines

BT474 cells (ATCC HTB20), a primary breast cancer cell line, and MDA-MB-231 cells (ECACC92020424), a metastatic breast cancer cell line, were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 1% bovine serum albumin (BSA) for 24 hours at a density of 30,000 cells/well in a 96-well plate. In some experiments cells were treated with TGFβ-1 at a concentration of 0, 2.5, 5.0, 7.5, and 10 ng/ml for a 24-hour period. Culture supernatants were removed and assayed for VEGF by ELISA. Cells were washed twice in PBS and lysed by freeze thawing. Total cellular protein was measured using the Coomassie assay (Pierce Biochemicals, Rockford, IL) according to manufacturer's instructions. VEGF levels were expressed as pg VEGF/μg cell protein. Data were analyzed by Student's t-test or ANOVA using DataDesk 4.1 (Data Description Inc., Ithaca, NY).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Cells were grown for 16 hours in DMEM with 1% BSA at a subconfluent density of 150,000 cells/well in a 12-well plate. Total RNA was isolated using Trizol (Gibco-BRL, Paisley, Scotland) according to the manufacturer's instructions. RNA (1 μg) was reverse transcribed at 37°C for 3 hours using 0.5 μg of random primers (Promega, Madison, WI) and 200U of Superscript II RT enzyme (Gibco-BRL). PCR amplification of VEGF cDNA was carried out by 30 cycles of 94°C for 1 minute, 55°C for 2 minutes, and 72°C for 1.5 minute using the following primers as described previously (9):