Clinical significance of serum vascular endothelial growth factor in advanced malignant tumors*

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Abstract  Objective: To elucidate the clinical significance of serum vascular endothelial growth factor (VEGF) level in patients with advanced cancer. Methods: Enzyme linked immunosorbent assay (ELISA) was used to determine the serum VEGF concentration in 40 patients with advanced cancer [non-small cell lung cancer (NSCLC), esophageal cancer (EC) and nasopharyngeal carcinoma (NPC)] before and after chemotherapy and 10 healthy volunteers as control group. Results: The serum VEGF concentrations in 40 cases of advanced cancer patients were significantly higher than those of 10 healthy control cases [(477.07 ± 374.10) pg/mL vs (139.09 ± 133.41) pg/mL; P = 0.016]. The serum VEGF concentrations in patients with NSCLC, EC and NPC were (518.53 ± 378.99) pg/mL, (399.21 ± 393.69) pg/mL and (500.68 ± 348.48) pg/mL, respectively. The differences were all statistically significant as compared with healthy control group (P values were 0.011, 0.044 and 0.019, respectively). The serum VEGF concentrations of the patients in response to chemotherapy was significantly lower than those of the same patients before they undergoing chemotherapy [(400.41 ± 332.84) pg/mL vs (777.10 ± 666.01) pg/mL; P = 0.034]. Conclusion: The serum VEGF level might be a novel and promising tumor marker of advanced malignancies and a predictor of disease progression, prognosis and therapeutic efficacy.

Key words  vascular endothelial growth factor (VEGF); advanced malignant tumor; serum

It has long been recognized that the development and metastasis of clinically relevant tumors is dependent on the recruitment and formation of new blood vessels to sustain growth. In microenvironment, tumor cells disrupt the normal balance of pro-angiogenic and anti-angiogenic forces by secreting growth factors responsible for stimulation of vessels. Hitherto, vascular endothelial growth factor (VEGF) has been reckoned as one of the key pro-angiogenic factors with strong biological effects of stimulating the proliferation and migration of endothelial cells (ECs), and increasing the permeability of blood vessel in order to provide sufficient oxygen and nutrients required for the growth of malignant tumors. Serum level of VEGF expression in cancer patients has been proved to have a tight association with the staging and tumor burden, furthermore, it also appears to have a close relationship with metastasis and prognosis in a variety of cancer types. This study aims to clarify the possible relationship between serum VEGF concentrations and disease progression as well as prognosis through the detection of serum VEGF levels in patients with advanced cancer before and after chemotherapy, and provide evidences for the prediction of clinical efficacy, the surveillance of disease progression and the adoption of therapeutic strategies.

Materials and methods

Subjects

Forty patients with histological or cytological confirmed advanced malignancies hospitalized in the Cancer Hospital of Shantou University Medical College (China) between May 2004 and April 2005, and 10 healthy control subjects were included in this study. The pathological types of cancers included: non-small cell lung cancer (21 cases), esophageal cancer (13 cases) and nasopharyngeal carcinoma (6 cases). The patients included 29 males and 11 females with a medium age of 53 (ranged from 27 to 74) years. All patients were required to provide written informed consent. This study was approved by the Cancer Hospital Ethical Committee of Shantou University Medical College (China).

Chemotherapy regimens

Patients with NSCLC were treated with NP regimen (Vinorelbine 25 mg/m², d1, d8 + Cisplatin 70 mg/m², d1) or GP regimen (Gemcitabine 1250 mg/m², d1, d8 +
Cisplatin 70 mg/m², d1). And patients with EC or NPC were all treated with HP regimen (Hydroxycapeothelin 6 mg/m², d1–3 + Cisplatin 70 mg/m², d1).

Collection of samples

Serum VEGF levels of the blood samples obtained from 40 patients with confirmed advanced malignancies before and after undergoing chemotherapy were measured with the quantitative sandwich enzyme-linked immunosorbent assay (ELISA, R&D system, USA). And 10 normal blood samples from healthy volunteers were taken as control. Blood samples were collected in evacuated blood collection tubes (B&D, USA) in a volume of 2.0 mL for each patient without adding any anti-coagulants. After centrifugation at a speed of 1000 r/min for 15 minutes, the supernatants of all the blood samples were stored at –80 °C before examination.

Laboratory examinations

Serum concentrations of VEGF were determined by commercial ELISA methods with Quantikine human VEGF kits (R&D Systems, Minneapolis, USA) by strictly following the instructions of the assay kit with a test protocol as follows: (1) Prepared all reagents, ELISA plates and standard sample; (2) Added 100 μL of RD1W diluting solution to all wells; (3) Added 100 μL of standard sample, control solutions and serum sample in each well, then covered the ELISA plate with a single layer of plastic wrap and incubated 2 h at room temperature (18 °C to 25 °C); (4) Prepared washing solution by adding 21 mL of wash buffer (25 x) to 500 mL double-distilled water few minutes prior to use; (5) Emptied and washed ELISA plate 3 times (each time for 3 minutes) with wash buffer, then discarded the fluid and dried the ELISA plate; (6) Added 200 μL VEGF-conjugate, then covered the ELISA plate with a single layer of plastic wrap and incubated 2 h at room temperature (18 °C to 25 °C); (7) Mixed color reagents A and B in a ratio of 1:1; (8) Repeated step 5; (9) Added 200 μL VEGF-conjugate, then covered the ELISA plate with a single layer of plastic wrap and incubate 25 min at room temperature (18 °C to 25 °C); (10) Added 50 μL stop solution to all wells including blank wells, then blanked microplate reader (STAT FAX-2100, USA) and measured color intensity at the wave length of 450 nm (630 nm as an alternative wave length) within 30 min; (11) Finally, recorded the values of optic density (OD) and the corresponding VEGF concentrations (pg/mL) of each well.

Statistical analysis

Statistical analyses were processed with SPSS 13.0 software. The results were presented as mean ± SD. Individual comparisons between two groups were performed by the Student t test and P < 0.05 was considered to be statistically significant.

Results

The serum VEGF concentrations of 40 advanced cancer patients were significantly higher than those of 10 healthy control cases [(477.07 ± 374.10) pg/mL vs (139.09 ± 133.41) pg/mL; P = 0.016]. The concentrations of VEGF in serum of patients with NSCLC, EC and NPC were (518.53 ± 378.99) pg/mL, (399.21 ± 393.69) pg/mL and (500.68 ± 348.48) pg/mL, respectively. The differences were all statistically significant as compared with healthy control group (P values were 0.011, 0.044 and 0.019, respectively; Table 1).

There were no statistical difference in serum VEGF concentrations of the 40 advanced cancer patients in different genders and ages. There were no statistical differences in VEGF concentrations of the 21 cases of NSCLC patients in different pathological subtypes, ages and genders (P > 0.05; Table 2).

There were 19 patients (10 of them with NSCLC) with clinical responses (PR + CR) in all groups, and 21 patients were with progressive, stable diseases or without receiving any treatment. The serum VEGF concentrations of samples from patients with clinical response significantly lower than those of the same group of patients before chemotherapy (P < 0.05). The concentrations of serum VEGF in patients in response to treatment were significantly lower than those of the same group of patients before chemotherapy [(400.41 ± 332.84) pg/mL vs (777.10 ± 666.01) pg/mL; P = 0.034; Table 3].

Discussion

Angiogenesis is a complex multistep process involving extensive interplay between cells, extracellular matrix components and soluble factors, such as VEGF, FGF, EGF, PDGF and TGF. VEGF appears to be one of the key angiogenic factors frequently used by tumors and other tissues to switch on their angiogenic phenotypes. In fact, nearly all tumors express VEGF at high levels. Previous studies show that VEGF-stimulated blood vessels are essential not only for primary tumor growth but also for metastasis. Bevacizumab, a monoclonal antibody against VEGF, has shown promising clinical activity against metastatic colorectal cancer, non-small cell lung cancer, breast cancer and renal cell carcinoma, especially in combination with chemotherapy. And the response rates and median durations of survival were both superior to chemotherapy alone.

Most tumor cells are capable of secreting VEGF, which can induce angiogenesis in tumor tissues, increase the vascular permeability, assist the entry of tumor cells into the vascular system and facilitate invasion and metastasis.