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Expression of VEGF and its effect on cell proliferation in patients with chronic myeloid leukemia

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Abstract. – OBJECTIVE: The aim of this study is to investigate the expression of vascular endothelial growth factor (VEGF) in the serum of patients with chronic myeloid leukemia (CML) and the effect of VEGF on cell proliferation.

PATIENTS AND METHODS: Serum VEGF levels in 12 CML patients (7 chronic phase, 5 blast crisis phase) were measured using enzyme linked immunosorbent assay (ELISA). VEGF expression was interfered by transfection of K562 cells. VEGF mRNA levels in transfected K562 cells were determined using RT-PCR and the effect of VEGF on the proliferation of transfected K562 cells was investigated.

RESULTS: VEGF expression levels were significantly higher in CML patients than normal controls and significantly increased during blast crisis phase than during chronic phase. Compared to controls, the proliferation of the K562 cells was suppressed when VEGF expression was inhibited. However, the inhibited proliferation of K562 cells after gene silencing of VEGF was partially abolished after introducing exogenous VEGF into the cells.

CONCLUSIONS: VEGF plays an important role in the initiation and development of CML and monitoring serum VEGF assists guiding the treatment and predicting the prognosis of CML.

Key Words:
Chronic myeloid leukemia, Vascular endothelial growth factor, Cell proliferation.

Introduction

Chronic myelogenous leukemia (CML) is a malignant clone disease of pluripotent hematopoietic stem cells characterized by myeloid cell hyperplasia accompanied by the presence of specific Philadelphia (Ph) chromosome and BCR/ABL fusion gene[1-3]. Recent studies[4] have shown that angiogenesis is closely associated with the development and prognosis of malignant blood diseases. Currently, vascular endothelial growth factor (VEGF) is considered as the most potent pro-angiogenic factor that plays crucial roles in the pathogenesis and development of tumors[5]. Bellamy et al[6] have demonstrated that VEGF is expressed in 12 types of cell lines such as leukemia, lymphoma and multiple myeloma cell lines, the amount of VEGF secreted by leukemia cells are markedly higher than that secreted by normal cells. VEGF promotes angiogenesis. In turn, the newly formed blood vessels enhance the cell division and proliferation of leukemia cells, thereby, promoting the progression of the disease[7]. Luo et al[8] have reported that VEGF depletion enhances sensitivity of arsenic trioxide in CML patients, indicating that higher expression of VEGF *in vivo* is associated with poorer prognosis of CML. However, to date, few studies have been conducted on the relationship between VEGF and CML. In the present study, CML patients were examined for the expression of VEGF and the influence of VEGF on cell proliferation was investigated by gene interference of VEGF.

Patients and Methods

Patients

Between May 2013 and May 2015, 12 CML patients (8 males, 4 females, an age range of 19-82 years, mean age 52 years) admitted to our institution were included in CML group. Of these patients, seven were confirmed to be at chronic phase and 5 were at blast crisis phase. The control group includes 12 normal persons (6 males, 6 females, age range of 26-73 years, mean age of 45 years) seeking health check-up at our institution during the same period.

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Sample Collection

Five milliliters of peripheral blood samples were collected from CML patients. Blood samples were allowed to stand at room temperature followed by centrifugation. The resulting supernatant were collected and stored at -20°C. Subsequently, VEGF expression was measured using ELISA with VEGF kit (R&A System, Minneapolis, MN, USA).

Reagent

Trizol RNA isolation kit was purchased from Gibco, Grand Island, USA. RT-PCR kit was purchased from Femantes, Big Cabin, OK, USA. PCR kit, DNA marker and DEPC were purchased from Sunshine Biotechnology, Nanjing, China.

Cell Transfection

K562 CML cell line (Institute of Biochemistry and Cell Biology, SIBS, CAS, China) were suspended and grown in Roswell Park Memorial Institute (RPMI)-1640 medium. The antisense oligonucleotides are complementary to bases 6-24 relative to the transcriptional start site of VEGF. The sequences were as follows: Antisense 5'-CACCCAAGACAGCAGAAAG-3'; Sense: 5'-CTTTCTGCTGTCTTGGGTG-3'. Cell transfection was performed using Lipofectamine 2000 (Invitrogen, Shanghai, China) and the efficiency of cell transfection was determined by using RT-PCR (Table I).

Generation of Cell Growth Curve

Cell suspension of logarithmic K562 cells adjusted to a cell density of 2×10^4/ml were seeded in 96 -well cell culture plates. Cell cultures were divided into three groups with three triplicates for each group. The study group was incubated with sense and antisense nucleotides respectively whereas the control group was incubated with RPMI-1640 with a final concentration of oligonucleotide at 4 μm. Cells were harvested at 24h, 48h, 72h and 96h after incubation. Cell viability was assessed using 0.4% trypan blue staining. Calculation was repeated for three times for each well to obtain mean value.

Transfection of Exogenous VEGF

Cell suspension of logarithmic K562 were seeded in 24-well culture plates at a cell concentration of 1×10^4/well. The cell cultures were incubated with 4 μm of antisense oligonucleotides and VEGF 165 (Peprotech EC LTD, purchased from Jinmei Bio-Engineering, Shanghai, China). As for control group, cells were incubated in RPMI-1640 and harvested after 48h. Cell viability was assessed using 0.4% trypan blue staining. Calculation was repeated for three times for each well to obtain mean value.

Statistical Analysis

Data analyses were performed using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). Quantitative data was expressed as mean ± SD. Differences between groups were analysed using two independent samples *t*-test. $p < 0.05$ was considered to be statistically significant.

Results

Serum Concentration of VEGF

Serum concentrations of VEGF in CML patients were significantly higher than those in normal controls (115.8 ± 32.72 (ng/L) *vs.* 391.95 ± 91.21 (ng/L), $p < 0.05$) (Table II), which indicated that VEGF expression was elevated in CML patients.

VEGF Levels During Chronic Phase and Blast Crisis Phase

Serum VEGF levels were determined in CML patients during different phases. Serum VEGF levels in CML patients during chronic phase were significantly lower than those in CML patients during accelerated phase (267.4 ± 58.89 (ng/L) vs. 473 ± 51.12 (ng/L), $p < 0.05$) (Table III).

Effect of Low Expression of VEGF on the Proliferation of K562 Cells

Compared with control group, VEGF mRNA levels were significantly decreased in K562 cells transfected with antisense oligonucleotides ($p <$

Table I. Primer sequences for RT-PCR.

Name	Forward (5′-3′)	Reverse (5′-3′)
VEGF	TCGGGCCTCCGAAACCATGA	CCTGGTGAGAGATCTGGTTC
β-actin	CGAGAAGATGACCCAGATCA	GATCTTCATGAGGTAGTCAG

Table II. Serum VEGF levels in CML patients and normal controls.

Group	No.	VEGF (ng/L)
Normal control	12	115.8 ± 32.72
CML patients	12	391.9 ± 91.2*

**Vs.* normal controls, $p < 0.05$.

Table III. VEGF levels during chronic phase and blast crisis phase of CML.

CML Staging	No.	VEGF (ng/L)
Chronic phase	7	267.4 ± 58.89
Blast crisis phase	5	473 ± 51.12*

**Vs.* chronic phase, $p < 0.05$.

0.01) (Figure 1). Meanwhile, along with the extension of incubation after transfection, cell proliferation was significantly reduced in these transfected cells than controls ($p < 0.05$) (Figure 2). However, no significant differences were observed in cell proliferation speed between the cells transfected with sense oligonucleotides and control group, indicating that low expression of VEGF can suppress the proliferation of K562 cells.

Effect of High Expression of VEGF on the Proliferation of K562 Cells

Introducing exogenous $VEFG_{165}$ into cells transfected with antisense oligonucleotides can partially abolish the inhibition of antisense oligonucleotides on K562 cell growth (Figure 3), with statistical significance ($p < 0.05$). This result further suggested that VEGF can promote cell proliferation.

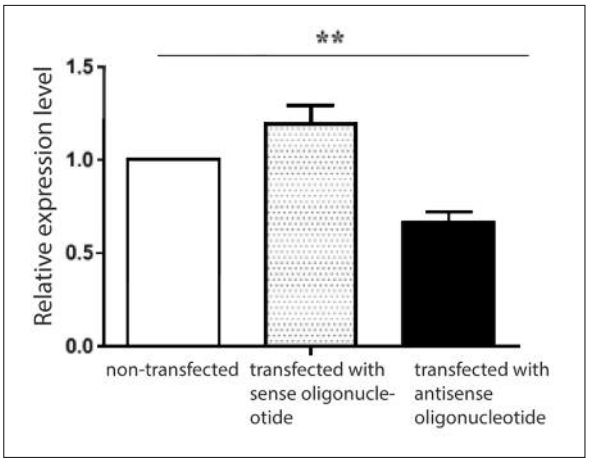

Figure 1. VEGF expression in K562 cells transfected with sense and antisense oligonucleotide.

Discussion

Malignancies of blood system, such as multiple myeloma and leukemia, as well as solid tumors present angiogenesis, which is strictly regu-

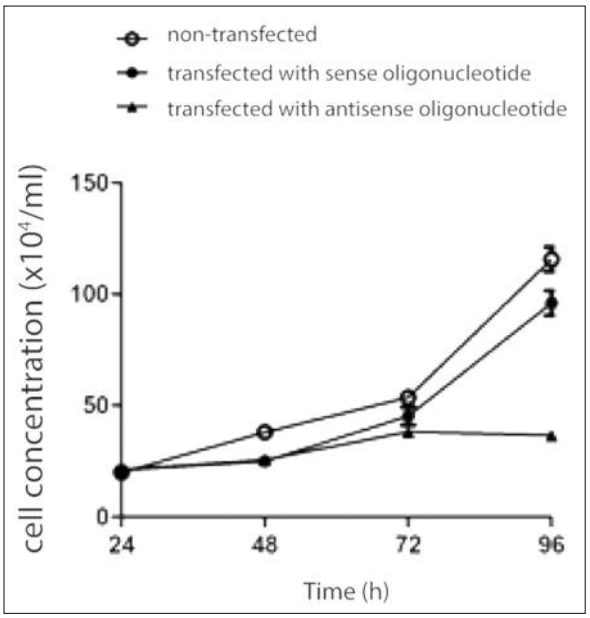

Figure 2. Effect of transfection with sense and antisense oligonucleotides on the growth of K562 cells at various time points.

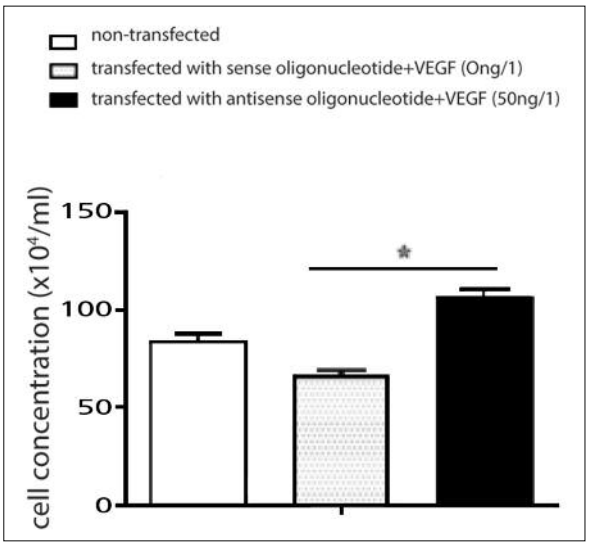

Figure 3. Effect of exogenous VEGF on the growth of K562 cells.

lated by both positive and negative regulatory factors. CML is a malignant disorder of blood system derived from pluripotent hematopoietic stem cells[9]. The clinical staging of CML is divided into chronic phase, accelerated phase and blast crisis phase. Once the disease is progressed to blast crisis phase, patients are not sensitive to treatment and result in shortened survival. Therefore, study on the pathogenesis of CML can help impeding the initiation and development of the disease as well as discovering novel therapeutic strategies.

VEGF, as an essential pro-angiogenic factor, plays an important role in the development of CML[10]. Legros et al[11] have revealed that VEGF secretion by bone marrow and plasma concentration of VEGF are significantly increased in newly diagnosed CML patients. Godoy et al[12] have shown that circulating endothelial cells (CECs) and VEGF are significantly higher in CML patients during different phases than healthy subjects. In addition, while VEGF expression is elevated in serum, the expression of corresponding receptors on cell surface, such as VEGFR-1 and VEGFR-2, are also increased. Studies[13] have demonstrated that VEGF in any form can activate VEGFR-1 and VEGFR-2 and, thereby, promote the proliferation of leukemia cells. This finding further reveals the critical role of VEGF in the initiation and development of CML.

The present study showed that serum VEGF levels in CML patients were significantly higher than those in normal population and VEGF expression was significantly higher during blast crisis phase than during accelerated phase, suggesting that VEGF may serve as a potential molecular marker for disease progression. Kaiafa et al[14] have mentioned that serum VEGF expression should be monitored during the treatment with dasatinib and can be used as a marker for predicting prognosis after the treatment. In addition, the effect of VEGF on the proliferation of K562 cells was investigated by interfering VEGF expression in K562 cells. The results showed that cell proliferation was significantly suppressed in the cells with low expression of VEGF than in controls. However, cell proliferation speed was partially restored after introducing exogenous VEGF into the cells with VEGF inhibition, indicating that higher levels of VEGF can promote cell proliferation.

Conclusions

VEGF plays an important role in the initiation and development of CML and monitoring serum VEGF assists guiding the treatment and predicting the prognosis of CML.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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