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Patterns of Vascular Endothelial Growth Factor Expression in Hematopoietic Malignant Cells

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ABSTRACT

Background and Objective: Vascular endothelial growth factor (VEGF) is a cytokine which is overexpressed in many malignant cancers including leukemia. VEGF plays an important role in tumor invasion and metastasis. Determination of the pattern of VEGF expression in human leukemic cell lines could be useful not only in screening of new antileukemic agents but also to study the mechanism of their action. In the present study, the pattern of VEGF production in some human leukemic cell lines has been assessed in vitro.

Materials & Methods: Three human leukemic cell lines (Molt-4, Jurkat and U937) were used in this study. The cells were cultured in complete RPMI medium and then incubated with different concentrations of phorbolmyristate acetate (PMA) or phytoheamagglutinin (PHA) for 48 hours. The level of VEGF secreted in the cellculture supernatants were measured with enzyme-linked immunosorbent assay kits (R and D systems).

Results: U937 cells produced a large amount of VEGF without any stimulus and PHA/PMA did not show any substantial effect on VEGF production by U937 cells. However, Molt-4 and Jurkat cells, produced VEGF less than U937 when cultured alone (with no stimulation) and PMA/PHA significantly increased the VEGF production in these cells dose-dependently.

Conclusion: Different patterns of VEGF expression were shown in different leukemic cell lines (Molt-4, Jurkat and U937) used in this study. These cells could be useful tools for screening of VEGF modulators and so for planning of new drugs for treatment of leukemia and other VEGF related disorders.

Key Words:
Vascular Endothelial Growth Factor
Leukemia

1. Introduction

Vascular endothelial growth factor (VEGF) is a cytokine which is produced by a number of cells and has a key role in angiogenesis (1,2). Angiogenesis, the procedure of new vessels development, plays an important role in tumor invasion and metastasis (3,4). VEGF is overexpressed in many malignant tumors (5,6). The increased VEGF level in serum of leukemic patients and also leukemia cell culture supernatants (7-9) have been reported. Also, a positive correlation between VEGF levels and malignant hematopoiesis has been revealed (10, 11). In this regard, an association between higher tumorigenicity and invasiveness of a human leukemic cell line in nude mice and high VEGF gene expression has been shown (12).

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Moreover, the relation between VEGF expression and multi drug resistance in leukemia has been suggested (13). Besides, the role of VEGF receptor in leukemic cells migration and proliferation has been shown (14, 15). In addition the induction of angiogenesis and inhibition of chemotherapy- induced apoptosis by recombinant VEGF-C (a member of VEGF family) in leukemic cells has been reported (16). Antiproliferative effect of soluble VEGF factor-1 in leukemic cells has been revealed in vitro (17). Furthermore anti-leukemic effects of some substances have been attributed to their inhibitory effect on VEGF expression and production (18, 19). Also the inhibitory effect of VEGF antisense oligonucleotide on VEGF mRNA expression and cell proliferation of human leukemic cells has been reported (20). Determination the pattern of VEGF expression in human leukemic cell lines could be useful not only in screening of new antileukemic agents but also in studying the mechanism of action of various environmental or nutritional materials with potential anti-cancer or anti- leukemic effects. In the present study the patterns of VEGF production in some human leukemic cell lines have been assessed in vitro.

2. Materials and Methods

2.1. Reagents

RPMI-1640 medium, penicillin, streptomycin, phytoheamagglutinin (PHA), phorbol myristate acetate (PMA) and trypan blue (TB) were purchased from Sigma (USA). Fetal calf serum (FCS) was from Gibco (USA). Microtiter plates, flasks and tubes were from Nunc (Falcon, USA). VEGF standard ELISA kit was obtained from R and D Company (USA).

2.2. Cell lines

Human leukemic monocyte [U937 (NCBI C130)] and T cells [Molt-4 (NCBI C149) and Jurkat (NCBI C121)], were obtained from NCBI (National Cell Bank of Iran, Pasteur Institute of Iran, Tehran). The cells were maintained in RPMI-1640 medium supplemented with 10% FCS and incubated in 5% CO2 at 37°C.

2.3. Cell culture and treatment

The method has been described in detail elsewhere (21). Briefly, the human leukemic cells were cultured in RPMI-1640 medium supplemented with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at 37°C in 5% CO2. The cells were seeded at a density of 10^6 cells/ml and prior to experiments were treated in fresh medium. Then, the cells were incubated with different concentrations of PMA (0-25 ng/ml) or PHA(0-10 µg/ml) for 48 hours The supernatants of cell cultures were collected, centrifuged and stored at -80°C for next experiments. All experiments were done in triplicate.

2.4. Evaluation of VEGF production by ELISA

The amount of VEGF secreted in the cell culture supernatants by human leukemic cell lines was measured with the Quantikine human VEGF ELISA kits (R and D systems) according to the manufacturer's instructions. This assay uses the quantitative sandwich enzyme immunoassay technique. Complete RPMI medium was used as control and human recombinant VEGF165 was employed as standard for drawing the standard curves.

2.5. Statistical analysis

VEGF level quantification in cell-conditioned media was performed in three independent experiments and the results were expressed as mean ± SEM. Statistical comparisons between groups were made by analysis of variance (ANOVA). $P<0.05$ was considered significant. Test of multiple comparison of Tukey was applied (5%) for statistically significant differences. The software SPSS 11.5 and Excel 2003 were used for statistical analysis and graph making respectively.

3.Results

3.1. Pattern of VEGF production in PMA-stimulated leukemic cell lines

Pattern of VEGF production in PMA- stimulated U937 cells

U937 cells produced a large amount of VEGF without any stimulus and PMA did not show any considerable effect on VEGF production by U937 cells as was shown in Figure 1. ($p<0.05$).

3.2. Pattern of VEGF production in PMA-stimulated Molt-4 cells

Molt-4 cells cultured alone (without any inducer), produced evident amount of VEGF production. PMA significantly increased VEGF production in Molt-4 leukemic cells dose- dependently as was shown in Figure 1. ($p<0.05$).

3.3. Pattern of VEGF production in PMA-stimulated Jurkat cells

Jurkat cells, showed noticeable production of VEGF when cultured alone (with no stimulation). PMA significantly increased the VEGF production in Jurkat leukemic cells dose-dependently as can be seen in Figure 1. ($p<0.05$).

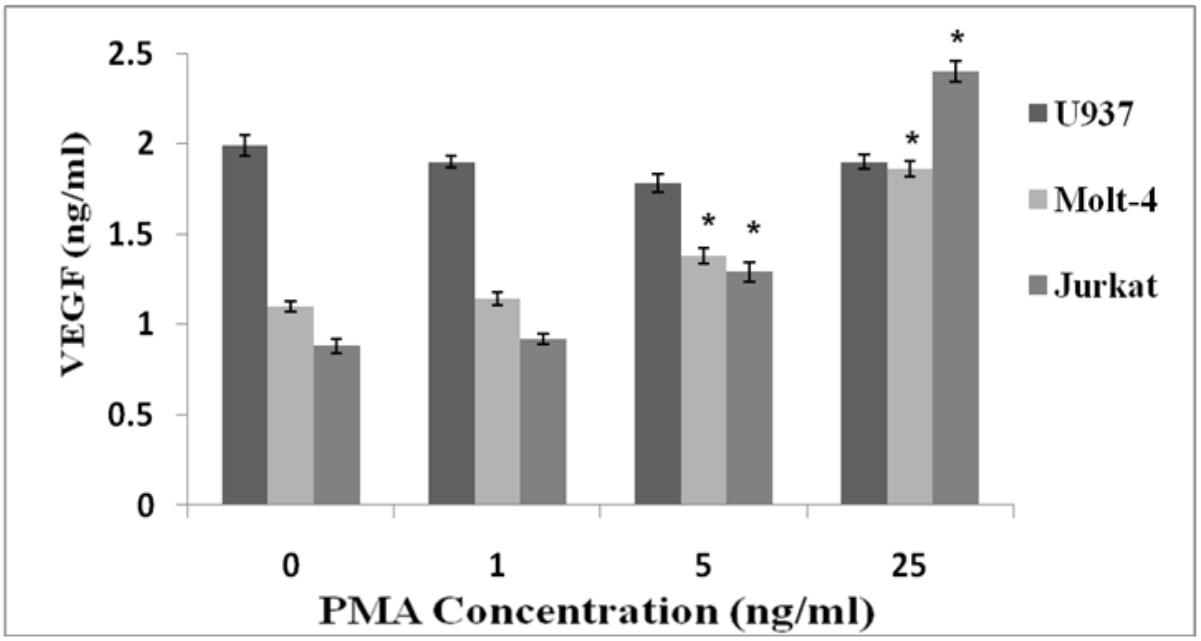

Figure 1: The effect of PMA on VEGF production by human leukemic cells

The U937, Molt-4 and Jurkat leukemic cells (1×10^6 cells/ml), distinctly, were cultured in complete RPMI-1640 medium and then were stimulated with different concentrations of phorbol myristate acetate (PMA) (0-25 ng/ml) for 48 hours. At the end of incubation, VEGF concentration in conditioned medium was measured by ELISA. Data are mean ± SEM of three independent experiments.* $p<0.05$ was considered significant.

3.4. Pattern of VEGF production in PHA-stimulated leukemic cell lines

Pattern of VEGF production in PHA- stimulated U937 cells

U937 cells produced a large quantity of VEGF without any stimulus and PHA did not show any substantial effect on VEGF production by U937 cells as was depicted in Figure 2. ($P<0.05$).

3.5. Pattern of VEGF production in PHA-stimulated Molt-4 cells

Molt-4 cells cultured without any inducer, showed noticeable amount of VEGF production

PHA significantly increased VEGF secretion in Molt-4 leukemic cells dose- dependently as was illustrated in Figure 2. (P<0.05).

3.6. Pattern of VEGF production in PHA-stimulated Jurkat cells

Jurkat cells, when cultured with no stimulus, showed obvious production of VEGF. PHA considerably increased the VEGF production in Jurkat leukemic cells dose- dependently as was represented in Figure 2. (p<0.05).

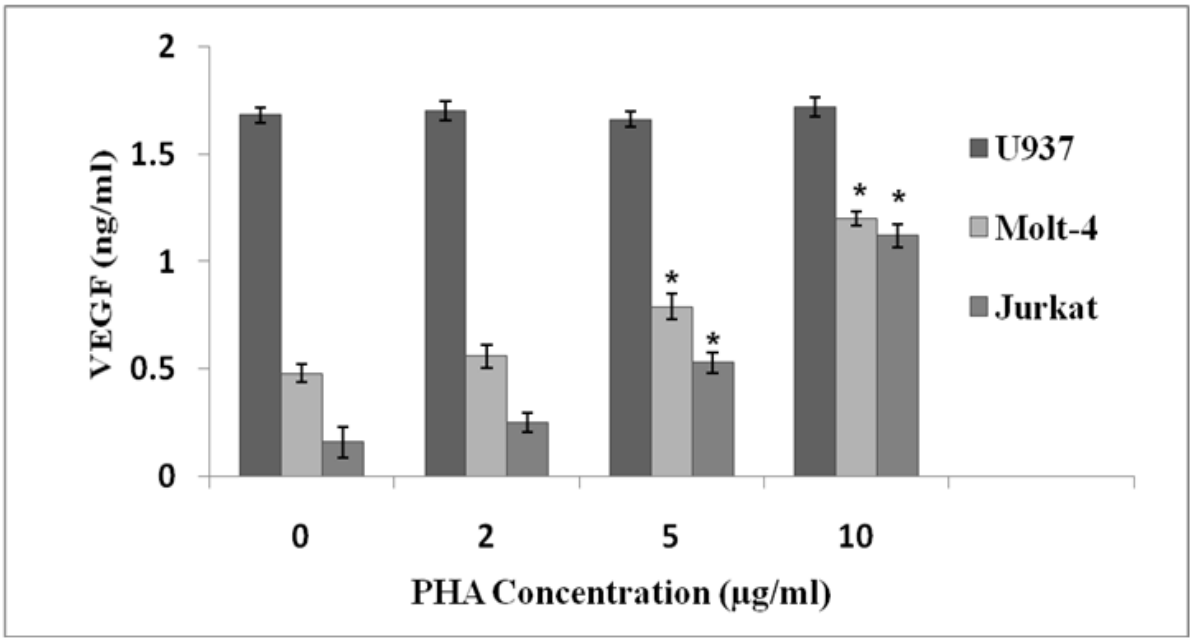

Figure 2: The effect of PHA on VEGF production by human leukemic cells

The U937, Molt-4 and Jurkat leukemic cells (1×10^6 cells/ml), distinctly, were cultured in complete RPMI-1640 medium and then were stimulated with different concentrations of phytoheamagglutinin (PHA) (0-10 µg/ml) for 48 hours. At the end of incubation, VEGF concentration in conditioned medium was quantified by ELISA. Data are mean ± SEM of three independent experiments.*P<0.05 was considered significant.

4. Discussion

In this study we found out that human leukemic U937, Molt-4 and Jurkat cell lines could potentially produce VEGF with different degrees. Different patterns of VEGF express in malignant and non-malignant tissues have been shown and different tumors show different profiles of VEGF expression (22, 23). VEGF plays an important role in tumor invasion and metastasis (24, 25) and is a key biomarker of malignancy (26). Thus VEGF could have prognostic and diagnostic value (27, 28). Some recent clinical researches have been focused on design and planning of VEGF inhibitors in order to avoid and control the tumor invasion and metastasis (29, 30). Determination the profiles of VEGF expression in particular tumors, could be valuable in cancer treatment (31, 32).

In this study, we used PMA and PHA as inducers of VEGF expression. Numerous studies have shown the induction or increase of VEGF expression in PMA-stimulated cancer and normal cells (33-35). According to the results of present study, U937 cells produced a large amount of VEGF without any stimulus and PMA/ PHA did not show any considerable effect on VEGF production by U937 cells. Whereas Molt-4 and Jurkat cells produced noticeable amount of VEGF and PHA/PMA significantly increased VEGF secretion in these two cell lines. Thus our results show that there are diverse patterns of VEGF production in leukemic cells and PMA/PHA has different effect on VEGF production in different leukemic cells. So sensitivity of various leukemic cells to PHA or

PMA-induced VEGF production is different. Consistent to us, Fusetti et al. (2000) reported that the leukemic Jurkat, Molt-4 and U937 cells produced different extents of VEGF (36). In Fusetti et.al study, the concentration of VEGF produced by leukemic cell lines was in this order: U937 > Molt-4 > Jurkat cells (similar to us). Nevertheless in Fusetti et.al study Jurkat cells produced VEGF with < 0.01 ng/ml concentration while in our study Jurkat cells (without any stimulation) produced VEGF with ~ 0.88 ng/ml concentration. The difference between our results and Fusetti et.al may be in part due to the number of cells used or duration of incubation time. Fusetti et al. used 3×10^5 cells/ml cultured for 3-days while in this study 10^6 cells/ml were cultured for 2 day (48 hours). Moreover in our study PHA/PMA significantly increased the VEGF production in both Jurkat and Molt-4 cells. However Fusetti et al. cultured the leukemic cells alone and did not use PHA or PMA for stimulation. In another study performed by Avramis et. al (37), U937 cells produced high amount of VEGF without any stimulation (similar to us). But contrasting to us in Avramis et. al study, the Jurkat/E6-1 cells did not express mRNA or protein of VEGF. This discrepancy between our results and Avramis et. al may be in part due to the number of cells and methods of study. Also in Broggini et. al study, secretion of VEGF receptor -1 by leukemic Molt-4 cells has been shown (38).

Production of VEGF in macrophages such as human THP-1 cell line, after stimulation with PMA, has been reported (39). In addition, the role of mitogen-activated protein kinases as key mediators of VEGF production has been shown (40). Moreover interference with mitogenic signal transduction pathways decreases the expression of VEGF (41).

Like to our findings, diverse patterns of angiogenic factors such as VEGF have been found in B-cell acute lymphocytic leukemia (B-All) compared to B-cell chronic lymphocytic leukemia (B-Cll) (42). Invasive properties of lymphoblastoid cell lines through stimulation of angiogenesis have been reported (43). So invasiveness of lymphoblastoid cell lines may be partly due to VEGF production by them. Moreover important role of angiogenesis in the pathogenesis of hematologic cancers have been suggested (37).

The distinct expression of VEGF in different tumors has selective worth as might be potentially helpful in disease prediction and setting up the more suitable therapy procedures in associated conditions. Targeting the VEGF may be precious in control of leukemic diseases and their pathogenesis. Accordingly, it was reported that VEGF release in AML cells is of particular importance as is useful in disease prognosis and assessing the cells sensitivity to chemotherapeutic agents (44). It seems that U937, Molt-4 and Jurkat cells provide valuable screening tools for anti-angiogenic drugs, VEGF enhancers/ inhibitors and also study of regulatory mechanisms of VEGF secretion. Furthermore the leukemic cell lines used in this study may be good models for study and planning the useful therapeutic approaches for unmanageable leukemias.

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