

Lactate Dehydrogenase, not Vascular Endothelial Growth Factor or Basic Fibroblast Growth Factor, Positively Correlates to Bone Marrow Vascularity in Acute Myeloid Leukemia

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Background: Angiogenesis has been extensively studied in acute myeloid leukemia (AML). Lactate dehydrogenase (LDH), a common biochemical marker for tumor burden and anaerobic glycolysis, is a poor prognostic factor for AML. Regulated by hypoxia-induced factor, both vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are responsive to cancer-related angiogenesis. To study the roles of serum LDH, VEGF and bFGF in AML angiogenesis, we investigated bone marrow vascularity in untreated AML patients, and analyzed its relationship to serum LDH, VEGF and bFGF levels.

Methods: Eighteen (11 males, 7 females; mean age, 57.7 years) *de novo*, untreated AML patients were enrolled. Bone marrow vascularity was evaluated by staining bone marrow core biopsy tissue with endothelial cell marker CD31 or CD34. Serum LDH was determined with the Wroblewski-La Due method. Serum VEGF and bFGF were determined with enzyme-linked immunoassay. The relationship of LDH, VEGF and bFGF level to bone marrow vessel numbers was examined by linear regression.

Results: Log LDH significantly correlated to AML bone marrow vascularity ($r=0.61$; $p=0.007$). VEGF and bFGF concentrations did not correlate with AML angiogenesis.

Conclusion: These results suggest that serum LDH, but not VEGF and bFGF concentrations, can be used as a simple parameter for predicting vessel formation in AML bone marrow. [*J Chin Med Assoc* 2006;69(11):534–537]

Key Words: acute myeloid leukemia, angiogenesis, basic fibroblast growth factor, lactate dehydrogenase, vascular endothelial growth factor

Introduction

Angiogenesis is a complex, multistep process by which new microvessels are formed from preexisting vasculature.¹ Hypoxia is the factor most responsible for this process.² Increased anaerobic glycolysis due to elevated tumor cell proliferation and metabolism is one of the most important physiologic responses to hypoxia. When tissue undergoes anaerobic glycolysis, lactate dehydrogenase (LDH) increases to accelerate conversion

of pyruvate to lactate, yielding energy.³ At the molecular level, hypoxia induces expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) for angiogenesis.² Increased angiogenesis has been observed in acute myeloid leukemia (AML) patients.⁴ Simple parameters for prediction of angiogenetic ability in AML remain unclear. We investigated the association of peripheral blood serum LDH, VEGF and bFGF with bone marrow vascularity in AML patients.

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Methods

Study subjects

Study subjects were 18 untreated, *de novo* AML patients (11 males, 7 females; mean age, 57.7 ± 18.2 years). The diagnosis of AML was determined according to criteria proposed by the French-American-British Cooperative Group in 1984.⁵ Patient enrollment was voluntary and with informed consent. This project was approved by the institutional review board of Taichung Veterans General Hospital (No. 940103/453).

Bone marrow vessel counting

After staining bone marrow core biopsy tissue with endothelial cell marker CD31 (1:1,600; Dako Inc., Carpinteria, CA, USA) or CD34 (1:500; Vector Laboratories Inc., Burlingame, CA, USA) (Figure 1), 2 of the authors separately assessed vessel number in each bone marrow biopsy slide with light microscopy (400 \times) in 5 areas containing the highest numbers of vessels, as described by Padro et al.⁴

LDH, VEGF and bFGF assays

All assays were determined in duplicate; the mean value was used in statistical analysis. LDH in blood sera was determined with the Wroblewski-La Due method kit (Wako Ltd., Osaka, Japan). VEGF and bFGF in blood sera were determined with quantitative sandwich enzyme human VEGF and bFGF immunoassay kits (R&D Systems Inc., Minneapolis, MN, USA). LDH levels are presented in IU/L, and VEGF and bFGF concentrations in pg/mL. The minimum detection limits for VEGF and bFGF were 9 pg/mL and 3 pg/mL, respectively.

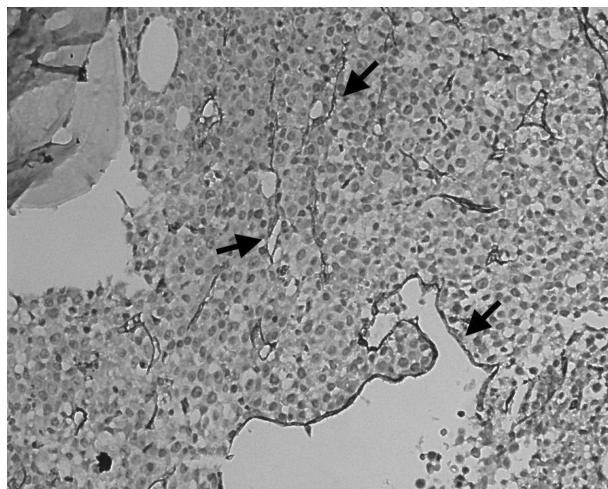


Figure 1. Immunohistochemical stain of bone marrow biopsy with CD31 showing brown-colored endothelial cells with vessel structure (arrows) (immunohistochemical stain with CD31, 400 \times).

Statistical analysis

The relationship of LDH, VEGF and bFGF concentrations to bone marrow vessel numbers and correlation among LDH, VEGF and bFGF were examined by linear regression. LDH was log transformed to improve similarity to normal distribution. Statistical analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). Significance was set at $p < 0.05$.

Results

Patients' basic data and original experimental results, including bone marrow vascularity, LDH level, VEGF and bFGF concentrations, are shown in Table 1. Vessel numbers in AML patients' bone marrow tissue were positively correlated to log peripheral blood serum LDH (Pearson's correlation coefficient, $r = 0.61$; $p = 0.007$) (Figure 2). VEGF data were not available for 2 patients due to insufficient specimens. Peripheral blood serum bFGF could not be detected in most of the patients. Bone marrow vascularity did not correlate to either peripheral blood serum VEGF or bFGF concentrations (VEGF: $r = 0.44$, $p = 0.86$; bFGF: $r = 0.22$, $p = 0.39$). There were no correlations among LDH, VEGF and bFGF concentrations.

Discussion

Angiogenesis has been extensively studied in AML,⁶ but simple parameters for predicting degree of AML angiogenesis are still absent. Our study demonstrated that serum LDH level is positively correlated to bone marrow vascularity in AML.

High LDH is a poor prognostic factor for several malignancies, including AML.⁷ Some possible mechanisms have been proposed. First, acidification of the extracellular water space by lactate and the subsequent activation of tumor invasion may be a rational explanation.⁸ Second, low pH microenvironment may increase cancer cell resistance to hypoxia-induced apoptosis by protecting mitochondria from oxidative stress.^{9,10} And third, overexpression of LDH, especially LDH5, reflects an upregulated hypoxia-induced factor pathway, which regulates glycolysis, angiogenesis, resistance to apoptosis, and even cancer metastasis.¹¹⁻¹³

Due to difficulty in counting leukemic tumor burden by tumor size or number of tumor cells, LDH is viewed as a simple biochemical marker for leukemic tumor burden prediction. Furthermore, Vidriales et al¹⁴ showed that serum LDH level was proportional to leukemic cells in synthetic phase. Also, bone marrow

Table 1. Patients' basic data and original experimental results

Patient	FAB classification	Sex	Age (yr)	Vessel no.	LDH (IU/L)	VEGF (pg/mL)	bFGF (pg/mL)
1	M3	M	29	37	617	84.8	<3
2	M0	F	36	19	269	80.2	<3
3	M2	F	50	9	373	38.7	<3
4	M2	M	66	18	172	127.3	<3
5	M2	M	69	21	403	134.6	<3
6	M2	M	70	28	832	101.1	<3
7	M2	M	29	24	302	91.6	4.2
8	M3	F	49	38	429	150.4	<3
9	M4	F	76	57	352	N/A	<3
10	M2	M	74	18	323	206.6	<3
11	M3	M	38	28	1,028	91.3	<3
12	M1	F	49	37	260	158.8	4.6
13	M4	M	75	20	474	158.1	3.5
14	M3	M	42	51	1,689	988.2	6.0
15	M2	F	82	30	929	N/A	<3
16	M4	F	58	55	4,621	214.1	<3
17	M4	M	61	25	321	46.4	<3
18	M2	M	86	19	363	538.2	<3

FAB = French-American-British; LDH = lactate dehydrogenase; VEGF = vascular endothelial growth factor; bFGF = basic fibroblast growth factor; N/A = not available.

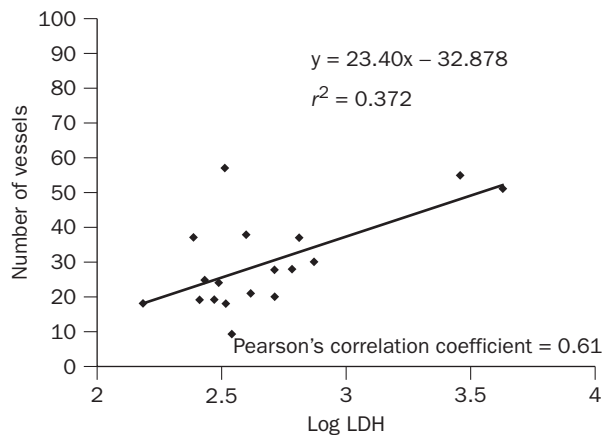


Figure 2. The number of vessels in acute myeloid leukemia bone marrow tissue is positively correlated to the log of blood serum lactate dehydrogenase activity (Log LDH) ($p=0.007$).

microenvironment plays an important role in elevated LDH in AML. Compared to other solid malignancies, AML obviously has a higher cell proliferation rate. Under rapid proliferation and immaturity of tumor cells, LDH is released due to multiple cytokine activity and cell membrane damage.¹⁵ The correlation between serum LDH and vascularity in AML bone marrow may reflect not only the degree of microenvironmental hypoxia, but also the degree of marrow involvement with leukemic cells, the aggressiveness of the leukemia, or the involvement of various cytokines.

As previously mentioned, LDH reflects an upregulated hypoxia-induced factor pathway. VEGF and

bFGF, positively regulated by hypoxia-induced factor, were also surveyed in this study. The roles of VEGF and bFGF in AML angiogenesis remain controversial. The importance of both VEGF and bFGF in AML angiogenesis was reported,^{16,17} but it has also been proposed that both VEGF and bFGF concentrations in AML patients are similar to those in healthy people.^{17,18} VEGF receptors, rather than VEGF itself, have prognostic value.¹⁸ A study by Bieker et al¹⁶ demonstrated that degree of bFGF expression in AML did not correlate with microvessel density. Our study confirmed their results, and further demonstrated the lack of correlation between serum VEGF level and AML bone marrow vascularity. Serum VEGF and bFGF were not good molecular markers of angiogenesis in AML.

VEGF and bFGF are currently known to be growth factors for hematopoietic cells, and not just angiogenic factors.^{19,20} The local effects of VEGF and bFGF on leukemic cell proliferation or angiogenesis may not be reflected in their peripheral blood concentrations. Further studies are required to answer this question.

We found a positive correlation between serum LDH level and bone marrow vascularity in AML patients, but no correlation of serum VEGF and bFGF levels with AML angiogenesis. These results suggest that serum LDH, but not VEGF and bFGF concentrations, can be used as a simple parameter in the evaluation of the angiogenic ability of AML. Anti-angiogenesis therapy is now rapidly developing in cancer treatment. Our findings may establish a simple indicator and

rationale that AML patients with high serum LDH level may be candidates for anti-angiogenesis treatment.

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