Chronic Lymphocytic Leukemia

Plasma levels of basic fibroblast growth factor and vascular endothelial growth factor and their association with IgV$_H$ mutation status in patients with B-cell chronic lymphocytic leukemia

The mutation status of genes encoding the variable region of immunoglobulin heavy chains (IgV$_H$) is a strong predictor of disease progression and survival in B-cell chronic lymphocytic leukemia (B-CLL). We investigated whether there is an association between the concentration of both vascular endothelial growth factor and basic fibroblast growth factor and IgV$_H$ mutation status in 49 untreated B-CLL patients.

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The overall survival of patients with B-cell chronic lymphocytic leukemia (B-CLL) is extremely heterogenous, so novel prognostic factors are being sought in order to identify high-risk patients at the time of diagnosis and to optimize their treatment. The mutation status of genes encoding the variable region of the immunoglobulin heavy chain (IgV$_H$) is currently considered one of key prognosticators of disease progression and survival.[1] Several studies have also shown that in B-CLL there is increased vascularity in bone marrow[2,3] and angiogenic cytokines in peripheral blood.[4-6] In this study, we investigated whether there is an association between concentrations of two key angiogenic activators, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) and IgV$_H$ mutation status in patients with B-CLL.

Forty-nine patients with never-treated B-CLL, diagnosed according to NCI-WG criteria, and a control group of fifty age-matched healthy blood donors were enrolled. IgV$_H$ mutation status was determined as described in detail elsewhere.[10] IgV$_H$ sequences were aligned to the nearest germline using the Ig BLAST program; IgV$_H$ genes with less than 98% sequence homology to the corresponding germline were considered mutated. We quantified bFGF and VEGF in EDTA plasma samples (stored at -70°C until the time of analysis) using sandwich ELISA kits (Human bFGF and VEGF Quantikine® Kit, R&D Systems, Minneapolis, MN, USA) according to manufacturer´s instructions. Software Analyse-It (Analyse-It Software Ltd., UK) was used for the statistical analyses. The non-parametric Mann-Whitney U test was used to compare differences between subgroups. The study was conducted according to Helsinki Declaration, approved by local ethics committee and study partcipants signed a written informed consent form. Twenty-six patients had mutated IgV$_H$ genes and 23 unmutated ones. The male:female ratio was 12:14 in IgV$_H$-mutated subgroup and 16:7 in the unmutated subgroup. The median of the IgV$_H$-mutated and unmutated patients was 58.2 and 62.3 years, respectively (95% CI [confidence interval], 57.5-67.1 and 57.8-62.6 years, respectively). According to modified Rai staging, 20, 5 and 1 IgV$_H$-mutated patients and 8, 12, and 3 IgV$_H$-unmutated patients had low, intermediate and high risk B-CLL, respectively. The concentrations of both VEGF and bFGF in peripheral blood plasma were significantly higher in B-CLL patients than in the control patients (p<0.0001 for both cytokines). The concentration of bFGF was significantly higher in both IgV$_H$ subgroups than in controls (p<0.0001) while VEGF was significantly increased only in IgV$_H$-mutated patients (p=0.0002); the difference between concentrations in controls and IgV$_H$-unmutated patients not being significant (p=0.0788, Table 1). Interestingly, the plasma levels of bFGF were significantly higher in the IgV$_H$-mutated group than in the IgV$_H$-unmutated subgroup (p=0.0149, Figure 1). On the other hand, VEGF concentrations in the mutated and unmutated subgroups were not significantly different (p=0.146). There was also no difference in VEGF or bFGF between patients with modified Rai low vs. intermediate vs. high risk disease. Likewise, no differ-

Table 1. Descriptive statistics of bFGF and VEGF levels in B-CLL patients and controls; results of Mann-Whitney tests.

Group	N.	Median	Mean	SD	95% CI of Mean	Mann-Whitney test	p value
bFGF Mutated	26	175.2	212.7	165.2	146.0-279.4	bFGF mutated vs. controls	<0.0001
bFGF Unmutated	23	46.3	91.7	98.0	49.3-134.0	bFGF unmutated vs. controls	<0.0001
bFGF Controls	50	8.9	11.0	10.3	8.1-13.9	bFGF mutated vs. unmutated	0.0149
VEGF Mutated	26	104.9	141.7	90.9	105.0-178.4	VEGF mutated vs. controls	0.0002
VEGF Unmutated	23	80.3	134.4	156.8	66.6-202.2	VEGF unmutated vs. controls	0.0788
VEGF Controls	50	49.0	68.4	63.6	50.4-86.5	VEGF mutated vs. unmutated	0.146

SD: standard deviation; CI: confidence interval. Concentrations are in pg/mL.

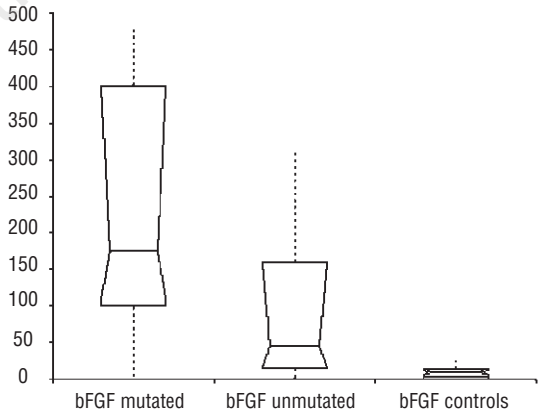

Figure 1. bFGF levels are significantly elevated in IgV$_H$ mutated patients. Concentrations are in pg/mL.

ence in either cytokine was seen between patients divided according to results of fluorescent *in situ* hybridization studies into a group with favorable cytogenetics (i.e. no abnormality or del 13q, n=30) and one with unfavorable cytogenetics (any other aberrations including del17p, 11q and +12, n=19) [*data not shown*].

As expected, plasma levels of bFGF and VEGF were significantly higher in the patients with B-CLL than in the controls; this is in agreement with previously published data.[5,6] Suprisingly, however, the concentration of bFGF was significantly elevated in patients with a favorable prognosis with mutated IgV_H genes while VEGF was raised in both mutated and unmutated cases. Plasma/serum concentrations of bFGF in CLL are by far highest of all hematologic malignancies[5] and increased bFGF in peripheral blood has been associated with an unfavorable disease course because of its correlation with advanced clinical stage,[7] increased survival of B-CLL cells[8] and enhanced resistance to fludarabine.[9] We hypothesize that our conflicting results could be caused by preferred usage of bFGF signaling by CLL cells in patients with IgV_H mutations patients due to different gene expression profiles. We cannot exclude bias caused by the relatively small number of samples and patient selection; on the other hand, some of the abovementioned studies investigating the role of bFGF in B-CLL also investigated limited numbers of patients' samples (3 in Koenig´s article,[8] 36 in Menzel´s study);[9] so all these results should be interpreted with caution. In conclusion, our data suggest a possible association between elevated bFGF and mutated IgV_H genes in B-CLL. Further investigation of the exact role of bFGF and VEGF signaling in B-CLL in larger series of patients, in particular with respect to modern prognostic factors, is clearly warranted.

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