

***bFGF* Polymorphism Is Associated with Disease Progression and Response to Chemotherapy in Multiple Myeloma Patients**

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Abstract. *Background/Aim: Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) play an important role in the initiation of angiogenesis. We aimed to assess whether polymorphisms within the genes coding for these angiogenic activators (VEGF (rs3025039;C>T) and bFGF (rs308395;G>C)) contribute to susceptibility and/or progression in multiple myeloma patients (MM) and to chemotherapy. Patients and Methods: One hundred and thirty-two patients with MM and 122 controls were genotyped for the VEGF and bFGF alleles by the PCR-RFLP technique. Genotyping results were compared regarding progression, risk of disease and response to treatment. Results: Patients in stage I-II disease (Durie-Salmon criteria) more frequently carried the bFGF -921G allele compared to patients in stage III (p=0.053) and healthy controls (OR=2.010, p=0.040). Progression after first-line chemotherapy was more frequent among patients carrying this variant (p=0.022). Conclusion: Our results imply that the course of disease in MM patients is associated with a polymorphism within the bFGF gene.*

Multiple myeloma is a plasma cell malignancy characterized by proliferation of clonal plasma cells in the bone marrow

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Key Words: VEGF gene polymorphism, bFGF gene polymorphism, multiple myeloma, disease susceptibility, response to immunomodulating therapy.

and secretion of monoclonal immunoglobulins detectable in the serum and/or urine. The process of angiogenesis has been proven to play a crucial role in the progression of multiple myeloma. Imbalance between antiangiogenic and proangiogenic factors (among them FGF - fibroblast growth factor and VEGF - vascular endothelial growth factor) can lead to the so-called "angiogenic switch", triggering vascular phase of the disease and inducing the clonal expansion of myeloma cells (1, 2). Evidence that VEGF expression is elevated in multiple myeloma has given rise to the use of antiangiogenic drugs in clinical practice (3).

The human *VEGF* gene is located on chromosome 6p21.3 and is organized into 8 exons separated by 7 introns (4). Several single nucleotide polymorphisms (SNPs) have been described in the *VEGF* gene, including a C to T substitution at position 936 (rs3025039) within the 3'-untranslated region (3'UTR), that is related to VEGF protein production (5). VEGF plasma levels were significantly lower in carriers of the 936T allele. Such observations could be attributed to the 936 C/T transition leading to loss of a potential binding sites for transcription factor AP-4 (activating enhancer binding protein 4).

The human *bFGF* gene is located on chromosome 4 (6). Polymorphisms within the promoter region of the *bFGF* gene may interfere with existing transcription factor binding sites or produce new binding sites and, therefore, influence *bFGF* gene expression (7).

In our previous studies, genetic variants of the *VEGF* and/or *bFGF* genes were found to contribute to the development of haematological malignancies – chronic lymphocytic leukaemia (CLL) (8) and non-Hodgkin's lymphoma (NHL) (9). In patients with NHL, the presence of the *VEGF* 936T allele was found to be associated significantly with worse prognosis of the disease, that was represented by a higher International

Prognostic Index (3-4 vs. 0-2 and 4 vs. 0-3 score, $p < 0.05$). The *bFGF -913G* variant was more frequently detected among NHL patients with aggressive histological subtype than those with indolent histological subtype and healthy individuals (9). In CLL, only a slight prevalence of the *VEGF T* variant was observed among patients as compared to healthy individuals, with a significant difference when high risk (stage III/IV) CLL patients were considered (8).

In the present study, a C to T substitution at position 936 within the 3'-untranslated region of the *VEGF* gene (rs3025039) and a C to G substitution at position -921 within the promoter region of the *bFGF* gene (rs308395) were analyzed in order to determine whether the presence of these allelic variants was associated with susceptibility and progression of the disease in patients with multiple myeloma. Patient response to immunomodulating therapy was also studied.

Patients and Methods

Patients. One hundred and thirty-two patients aged 37-85 years (median age=61), diagnosed with multiple myeloma, were included into the study. There were 70 men and 62 women. In 15 cases diagnosis of multiple myeloma was preceded by MGUS (monoclonal gammopathy of undetermined significance). 20 patients had renal insufficiency. Clinical characteristics of patients are presented in Table I. In addition, 126 healthy individuals of both sexes (M/F=63/63) served as a control group for the SNP study that involved 132 patients. Research was carried out in compliance with the Helsinki Declaration. The approval of Bioethical Committee of Wrocław Medical University was obtained for the study. Informed consent was obtained from all individual participants included in the study.

Immunomodulating therapy and response.

Thalidomide. Ninety-four patients were treated with thalidomide-containing regimens (81% with CTD: cyclophosphamide, thalidomide, dexamethasone). Response to first line therapy in patients was as follows: complete remission (CR) 14 patients, very good partial response (VGPR) 12 patients, partial response (PR) 41 patients, minor response (MR) 6 patients, stable disease (SD) 14 patients, progressive disease (PD) 7 patients. In 48 patients, megachemotherapy supported by autologous stem cell transplantation (autoBMT) was applied after first line therapy. Responses after autoBMT were as follows: 17 CR, 20 VGPR, 11 PR. Median progression free survival after induction chemotherapy was 14 months (range=0-60 months). We observed 20 cases of thalidomide-induced neuropathy and 2 cases were complicated by a thrombotic event while on thalidomide.

Lenalidomide. 27 patients out of the whole analyzed population were treated with lenalidomide-dexamethasone (Len-Dex) regimen, and for majority of them it was a third line therapy. Median number of cycles administered was 7 (range=2-35 cycles). In 17 patients neutropenia grade 6 was reported. Therapy was complicated by thrombosis in one patient. Responses to lenalidomide were as follows: 3 cases of CR, 6 cases of VGPR, 3 PR, 5 SD and 4 patients progressed while on lenalidomide. Median progression free survival was 5 months (range=3-31 months).

VEGF and bFGF genotyping. Ninety-six and 132 patients were typed for *VEGF* and *bFGF* polymorphism, respectively. One hundred and twenty-three healthy individuals served as a control group. The *VEGF* and *bFGF* alleles were detected using a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay as previously described (8, 9).

Statistical analysis. Genotype and allele frequencies were compared between the study groups by the Fisher's exact test. The odds ratio (OR) was calculated by Haldane's modification of Woolf's method and the significance of its deviation from unity was estimated by Fisher's exact test. Survival analyses were performed employing Kaplan-Maier analysis and log rank test. Probability values $p < 0.05$ were considered statistically significant, and those between 0.05 and 0.1 as indicative of a trend.

Results

Distribution of alleles and genotypes of VEGF and bFGF encoding genes in multiple myeloma patients and controls. The *VEGF* rs3025039 and *bFGF* rs308395 alleles and genotypes segregated similarly in patients with multiple myeloma and healthy individuals. None of the polymorphisms studied thus appeared to be associated with susceptibility to the disease. The *VEGF* CC homozygous genotype was the most frequent and was carried by over 80% of individuals in both groups tested, 83 out of 96 (86.5%) patients and 101 out of 122 (82.8%) healthy individuals. The *VEGF T* variant was present in 13 (3 homo-, 10 heterozygotes) out of 96 patients and 21 (including 8 homozygotes) out of 122 healthy individuals.

Among 132 multiple myeloma patients genotyped for the *bFGF* alleles, 99 (75.0%) patients were CC homozygotes, 31 (23.5%) were heterozygous for the *bFGF* alleles, and 2 (1.5%) were carrying the GG homozygous genotype. Ninety-nine (80.5%), 23 (18.7%) and 1 (0.8%) out of 123 healthy individuals were carrying the *bFGF* CC, GC and GG genotypes, respectively. The minor allelic frequencies were as follows: for the *VEGF 936T* it equalled 0.083 and 0.119, for patients and controls, respectively, and for the *bFGF -921G* 0.133 and 0.102, respectively.

Distribution of the *VEGF* and *bFGF* alleles and genotypes in controls closely resembles that observed in other Caucasian populations (described by Beránek *et al.* (7), Galimberti *et al.* (10), Kariz *et al.* (11), Petrovic *et al.* (12)). The *VEGF* CC homozygous genotype was overrepresented among patients with multiple myeloma (86.5%) as compared to patients with NHL (78.2%, $p=0.080$) and patients with CLL (70.6%, $p=0.017$) analyzed in our previous studies (8, 9). On the other hand, the frequencies of the *bFGF* alleles and genotypes in multiple myeloma patients did not significantly differ from those previously observed in CLL and NHL patients.

Interestingly, it was found that patients presenting with stage I-II of the disease (according to the Durie-Salmon criteria) more frequently carried the *bFGF -921G* allele as

Table I. Patient characteristics.

Data	N
Gender (n=132)	
Male	70
Female	62
Age (n=120)	
≥50 years	102
<50 years	18
Stage according to Durie-Salmon criteria (n=117)	
I	25
II	48
III	44
International staging system (n=117)	
I	39
II	42
III	36
Myeloma type (n=119)	
IgG	68
IgA	31
Light chain disease (LCD)	15
Non-secretory	4
Solitary	1
Beta-2-microglobulin (n=112)	
≤norm (≤1.80 mg/l)	12
>norm (>1.80 mg/l)	100
Anaemia (n=114)	
<10 g/dl	34
≥10 g/dl	80

compared to patients in stage III multiple myeloma and healthy controls (Figure 1A). Among 73 patients in stage I-II, 24 were carrying the G allele as compared to 7 out of 44 patients in stage III (0.33 vs. 0.16, $p=0.053$) and 24 out of 123 healthy individuals (OR=2.010, $p=0.040$). Moreover, the *bFGF* -921G allele was observed more frequently in patients with lambda light chains of IgA and IgG than in those with kappa chains (13/30 vs. 13/69, $p=0.014$).

Genetic variability within the VEGF and bFGF genes and response to immunomodulating therapy. The presence of the *bFGF* -921G allele was found to be associated with worse response to immunomodulating therapy. Progression of the disease after first line therapy was more frequent among patients carrying this allelic variant (6/32 vs. 4/88, $p=0.022$, Figure 1B). Similar relationships were seen in subgroups of patients stratified with respect to the chemotherapeutic regimens used, although not all of these relationships reached statistical significance.

In patients treated with the CTD regimen (cyclophosphamide, thalidomide, dexamethasone), presence of the *bFGF* -921G allele tended to be more frequent among those whose disease progressed after therapy (4/20 vs. 3/56,

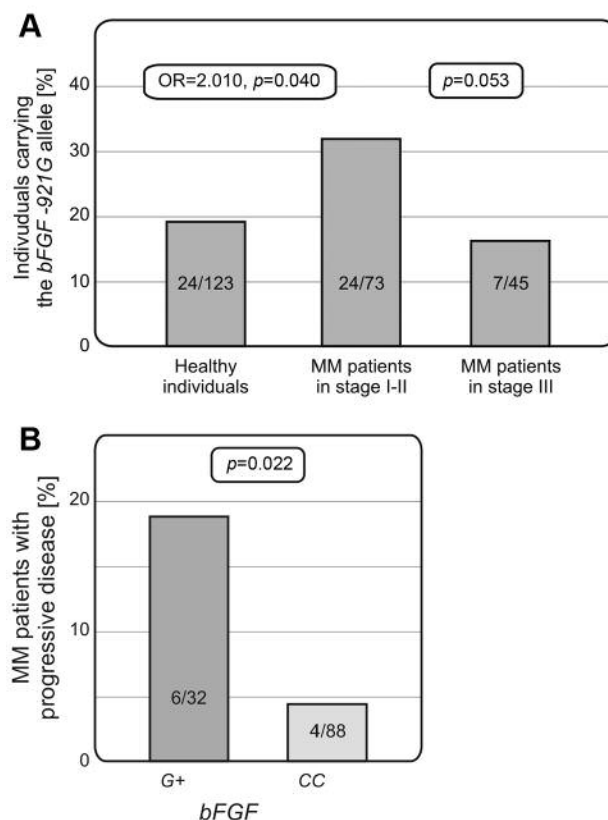


Figure 1. Associations of the *bFGF* (rs308395; -921 C>G) polymorphism with disease severity (A) and response to therapy (B). Stage III of multiple myeloma and progression of the disease after first line chemotherapy is more frequent among patients carrying the *bFGF* -921G allelic variant.

$p=0.073$) and in the group treated with MP/MPT/MPV (M-melphalan, P-prednisone, T-thalidomide, V-bortezomib) there were more individuals positive for the *bFGF* G allele among those who poorly responded to the administered therapy (stable or progressive disease; 2/4 vs. 0/12, $p=0.050$) compared to those with CR, VGPR or PR. We also observed that among patients treated with thalidomide (alone or in combination with other agents), the *bFGF* -921G allele was more common in patients who progressed after treatment (4/24 vs. 3/69, $p=0.070$). Although the above-mentioned relationships are not considered significant, they might indicate that the therapy with thalidomide is affected by the *bFGF* polymorphism.

The effect of the *bFGF* polymorphism on the lenalidomide therapy was also assessed. Among 27 patients subjected to lenalidomide-dexamethasone (Len-Dex) regimen, 8 were carrying the *bFGF* CG heterozygous genotype, while 19 were wild type homozygotes (with the CC genotype). Treatment related neutropenia was observed among 3 out of 6 patients with the G allele and only in 4 out of 11 CC homozygotes

(0.50 vs. 0.27, ns). Patients carrying the *bFGF* -921G allele responded less favourably to Len-Dex regimen. Progression of the disease after treatment was more frequent among carriers of the *bFGF* G allele (2/4 vs. 4/17, ns).

No significant association was observed for the *VEGF* polymorphism and response to treatment. No relationship was detected between polymorphic variants of the *VEGF* and *bFGF* genes and survival.

Discussion

The present study aimed to assess the potential association between the *VEGF* (rs3025039; 936 C>T) and *bFGF* (rs308395; -921 C>G) polymorphisms and the risk of multiple myeloma. None of the polymorphisms studied was found to be associated with susceptibility to the disease. However, presence of the *bFGF* CC wild-type genotype appeared to predispose patients to develop more advanced (stage III according to the Durie-Salmon's criteria) disease. This relationship has not been previously described.

Similarly to our results, a previous study by Faber *et al.* (13) showed that there are no associations between the *VEGF* polymorphism and multiple myeloma risk. However, in their study, the *VEGF* 936CC wild type homozygosity contributed to the risk of multiple myeloma in combination with either the *GSTM1* undelleted genotype or *GSTM1* undelleted + *GSTT1* null combined genotypes. Both these genes, the *GSTM1* and the *GSTT1*, encode the glutathione S-transferase (GST) enzymes. Thus, it seems that there is no direct association of the *VEGF* (rs3025039; 936 C>T) single nucleotide polymorphism (SNP) with the predisposition to multiple myeloma, although other SNPs within the *VEGF* gene have been implicated in multiple myeloma (14). Brito *et al.* found the higher frequencies of the *VEGF*-2578CC homozygosity and -2578CC/-634GG, -2578CC/-1154GG/-634GG *VEGF* genotype combinations in patients than in controls, associated with a 1.89-, a 5.52-, and a 4.91-fold increased risk for multiple myeloma, respectively. Moreover, they also observed that *VEGFR2* polymorphism may contribute to the risk of multiple myeloma. In their study *VEGF* -2578CC plus *VEGFR2* +1192GG, *VEGF* -2578CC plus *VEGF* -634GG plus *VEGFR2* +1192GG, and *VEGF* -1154GG plus *VEGF* -634GG plus *VEGFR2* -604TT combined genotypes were more frequently detected in patients than healthy individuals.

Furthermore, having analyzed 4 SNPs within the *VEGF* gene (-2578C>A (rs699947), -460C>T (rs833061), +405G>C (rs2010963) and +936C>T (rs3025039)), Andersen *et al.* (15) reported a possible relation between the haplotype -2578A/-460C/+405G (ACG) and the effects of thalidomide treatment. Patients with no copies of the ACG haplotype had a significantly longer time to next treatment than patients with one or two copies of this haplotype. However, no association

with the +936C>T (rs3025039) SNP was observed in that study, which is in agreement with our results.

There have been a number of studies that aimed to assess the effect of various gene polymorphisms on the therapy of patients with multiple myeloma (summarized in Vangsted *et al.* (16)) These included analyses of several SNPs in genes encoding molecules involved in DNA repair, apoptosis, inflammation or function of the nervous system. The influence of cytochrome *P450* 2C19 gene variations has also been studied and the effect was seen in Asian populations but not in Europeans (17-19).

To our knowledge, none of the previous studies considered the role of SNPs within the *bFGF* gene in the response to treatment in patients with multiple myeloma. We found that the *bFGF* -921 C>G (rs308395) SNP might be of prognostic value for the patients with multiple myeloma treated with thalidomide, although our results did not reach statistical significance on this. It should, however, be noted that an earlier study showed that thalidomide and bFGF interact somehow, probably indirectly, as the bFGF-induced cell proliferation and VEGF expression were reduced after thalidomide treatment (20). Also of note, a recent study showed that expression of VEGF and bFGF was significantly different in patients responding to IMiD-based therapy than in those who did not respond to that therapy (21). We observed that progressive disease was more frequent in patients carrying the *bFGF* -921 G allele.

These results suggest that the *bFGF* G allele might be a potential marker associated with worse response to therapy in patients with multiple myeloma. However, these data need to be verified in more extended studies involving numerous groups of patients.

Conflicts of Interest

The Authors declare no conflicts of interest.

Acknowledgements

This work was supported by statutory activity of Wrocław Medical University (Project No. ST-370).

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Received December 19, 2016

Revised March 8, 2017

Accepted March 9, 2017