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ORIGINAL ARTICLE

Increased expression of platelet derived growth factor receptor β on trephine biopsies correlates with advanced myeloma

Antonios Bilalis[1], Evi Pouliou[2], Maria Roussou[3], Asimina Papanikolaou[2], Anna Tassidou[2], Theophanis Economopoulos[1], Evangelos Terpos[3]

[1]Second Department of Internal Medicine-Propaedeutic, Athens University Medical School, Attikon University Hospital, Athens; [2]Department of Hematopathology, Evangelismos General Hospital, Athens; [3]Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece

Summary

Purpose: *Multiple myeloma (MM), a major cause of cancer mortality, is considered the second most frequent haematological malignancy in Europe. Angiogenesis is a multifactorial process that drives the tumorigenesis in solid tumors and in MM. The platelet derived growth factor (PDGF) receptors are cell surface tyrosine kinase receptors and play an important role in angiogenesis, cancer cell proliferation and dissemination. Few studies have been conducted regarding the expression of PDGF receptors and the correlation with clinical-pathological parameters and prognosis in MM. The purpose of our study was to evaluate, for the first time, in a large cohort of newly-diagnosed MM (NDMM) patients, the expression of PDGF receptor a and β (PDGFR a, β) in bone marrow trephine biopsies and investigate the association of PDGFR a, PDGFR β with angiogenesis in the bone marrow, assessed by bone marrow microvessel density (MVD), clinical characteristics and prognosis.*

Methods: *In this retrospective study, we assessed the relation of PDGFR a and PDGFR β immunohistochemical expression with MVD in formalin-fixed paraffin-embedded bone marrow sections from 120 NDMM patients. The immunoreactivity of PDGFR a and β was examined on the basis of positive plasma cells (PCs) with specific cut off values.*

Results: *PDGFRa and PDGFRβ were frequently expressed on malignant PCs. We found that increased PDGFRβ expression was strongly associated with advanced disease and adverse prognosis. The expression of PDGFRa and MDV were not correlated with specific features.*

Conclusion: *This analysis showed highly expressed PDGFRa and β PCs of NDMM patients and indicated that high PDGFRβ expression at diagnosis was associated with advanced-stage disease.*

Key words: *angiogenesis, immunohistochemistry, multiple myeloma, platelet derived growth factor receptor*

Introduction

MM is considered a common malignancy as it comprises the 1.5% of all malignancies and is the second most frequent haematological malignancy in Europe and USA with an age-adjusted incidence of 6 per 100000 per year [1]. With the improvements in the therapeutic management with the use of novel agents the prognosis of MM patients has increased but the disease remains incurable with the median survival of 6 years [2]. The introduction of new molecules (daratumumab, ixazomib, carfilzomib, elotuzumab, panobinostat, pomalidomide) in the therapeutic armamentarium of MM is considered that it could offer additional improvements for the management of the disease and the prolongation of patient survival [3-8]. MM is characterized by proliferation of clonal plasma cells in the bone marrow. The clinical manifestations of MM include hypercalcemia, renal failure, anemia, lytic bone lesions and immunodeficiency and some of them have prognostic value [9-11].

Correspondence to: Antonios Bilalis, MD. Second Department of Internal Medicine-Propaedeutic, Athens University Medical School, Attikon University Hospital, 12482, Athens, Greece.
Tel: +30 6972881945, E-mail: agbilalis@yahoo.gr
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Among the most important and frequently described parameters in the pathogenesis of MM is the role of bone marrow microenvironment which includes bone marrow stromal cells, cytokines, growth factors and growth factor receptors [12]. The bone marrow microenvironment in MM is characterized by an increased MVD and increased angiogenesis. In addition, there are several observations that support a direct correlation between MM progression and increased angiogenesis. Angiogenesis is a complex and multifactorial process that drives tumorigenesis in many solid tumors and in MM [13]. Among the growth factors and cytokines that play important role in the bone marrow microenvironment and angiogenesis are the PDGFR AB and its receptors α and β [14]. Evidence from the assessment of PDGFR α and β in the serum of MM patients shows strong positive correlation of these factors with the angiogenesis and MVD [15]. Furthermore, both PDGFR α and β have prognostic value in terms of overall survival, as higher PDGFR is associated with significantly higher MVD which is correlated with lower survival [15]. There has been continuous attempts to explore and identify pathways, growth factors, surface proteins or, in a word, targets that may play an important role in the facilitation of MM proliferation and that eventually may be in the focus for the development of specific targeted molecules like monoclonal antibodies, check point inhibitors and -in our study- tyrosine kinase inhibitors (TKIs). It is known that PDGFR isoforms and their receptors share functions that play a role in the regulation of growth and survival of certain cell types and overactivity of PDGFR signaling, by overexpression or mutational events, which may drive tumor cell growth [16]. PDGFR isoforms exert their cellular effects by binding to α and β tyrosine kinase receptors (PDGFR α and PDGFR β, respectively). The two PDGFR are structurally similar and consist of extracellular domains with 5 immunoglobulin-like domains and intracellular parts with tyrosine kinase domains. Ligand binding occurs mainly to Ig-like domains 2 and 3, and causes dimerization of the receptors [17]. The dimerization is a key event in activation since it brings the intracellular parts of the receptors close to each other promoting autophosphorylation between the receptors, a fact that initiates the signal-transduction cascade.

Methods

Patients

Our study was based in the retrospective evaluation of NDMM patients who had undergone trephine biopsy before the commencement of any type of treatment for MM. Assessed were formalin-fixed, paraffin-embedded bone marrow biopsies (BMB) of 120 consecutive patients with active symptomatic MM. Bone marrow trephine biopsies were paraffin-embedded and decalcified with low PH EDTA buffer (EDTA/HCL). Bone marrow sections from patients with chronic myeloproliferative neoplasms were used as positive controls, while sections from human tonsil were used as negative controls.

The study was conducted according to the Declaration of Helsinki guidelines and after approval from the Ethics Committees of our hospitals.

Evaluation of PDGFR α expression on plasma cells in trephine biopsies

Immunohistochemical staining was performed in an automatic instrument (DAKO Autostainer Link 48) using an anti-PDGFR β polyclonal antibody (P-20): sc-338 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and at 1:100 dilution. The immunoreactivity of PDGFR β was examined on the basis of positive PCs with the following cut off values: <20% positive PCs (negative group, group I), 20-40% positive PCs (intermediate expression group, group II) and >40% positive PCs (high expression group, group III).

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Evaluation of microvessel density on plasma cells in trephine biopsies

Immunohistochemical identification of endothelial cells was also performed in the bone marrow specimens that were fixed in buffered formalin, decalcified with EDTA/HCl and embedded in paraffin with a human monoclonal antibody against CD34 (DAKO A/S, Glostrup, Denmark) and VEGF (DAKO A/S, Glostrup, Denmark) by two experienced pathologists at ×400 magnification. In each biopsy sample, microvessels were counted in at least 3 independent hot spots per section and the MVD of a bone marrow biopsy was calculated as the mean value of all independent readings and recorded as the number of microvessels per ×400 field. The opinion variability between the two pathologists for the microvessel counts was < 5.0% (± 3.5%). For the microvessel count of 1-2 the angiogenesis was characterized as low grade, while intermediate grade angiogenesis was defined as the presence of a microvessel count of 3-6 and high grade angiogenesis as the presence of microvessel count of ≥7 [18].

Statistics

Data were expressed as mean±SD for quantitative variables and as percentages for qualitative variables. The Kolmogorov-Smirnov test was utilized for normality analysis of the quantitative variables. Bivariate analyses were made by using the Independent samples t-test or Mann-Whitney U test in case of violation of normality and chi-square test , Fisher's exact test to analyse the relation between the variables of interest and the quantitative , qualitative, demographic and clinical characteristics, respectively. All demographic or clinical variables with a p value <0.2 in bivariate analyses were included in a multivariate Cox regression model to determine the impact of variables of interest on overall survival and progression-free survival (PFS) after adjustment for potential confounders clinical and demographic variables. All tests were two-sided, and a p value <0.05 was used to denote statistical significance. All analyses were carried out using the statistical package SPSS version 17.00 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, Ill., USA).

Results

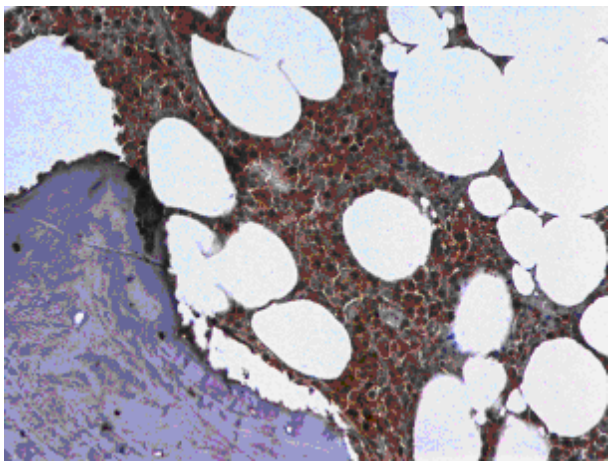
One hundred and twenty patients with newly NDMM were studied (74 males and 46 females). The median patient age was 67 years (range: 31-85). Sixty seven patients (56%) had ECOG performance status 0 and 1 and the rest >2. Fifty four patients (45%) had advanced disease at diagnosis (stage III according to ISS). Patient characteristics are shown on Table 1.

Table 1. Patient and disease characteristics

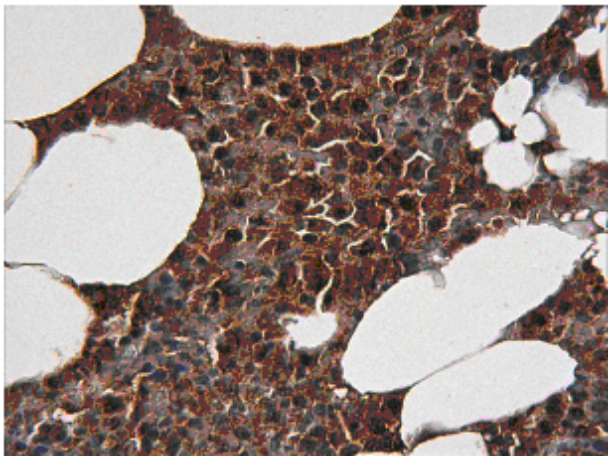
Characteristics	*Patients. n (%)*
Gender	
Male	74 (62)
Female	46 (38)
Age, years, median (range)	67 (31 – 85)
ECOG PS	
0,1	67 (56)
>2	53 (44)
Multiple myeloma type	
IgG	68 (57)
IgA	29 (24)
Light-chain	23 (19)
International Staging System (ISS)	
Stage I/ II	66 (55)
Stage III	54 (45)
Bone marrow plasma cells (%)	
<50	37 (31)
>50	83 (69)
Genetic lesions (in the 72 patients tested)	
t(11;14)	(18)
t(4;14)	(9)
t(14;16)	(6)
del(13q)	(34)
del(17p)	(12)
1q+	(21)

PDGFR a expression in bone marrow biopsies

The immunostaining for PDGFR α in bone marrow biopsies showed that 44 (37%) patients were negative (<20%) for PDGFR α expression (group I), while 28 (23%) had intermediate expression (group II) and 48 (40%) had a high PDGFR α positive expression on their PCs (group III; Figure 1). In our analysis we noticed that the PDGFR α was not correlated with important parameters such as PFS, disease stage as per ISS, and the clinical response, but in the multivariate analysis a significant correlation was revealed with markers that are connected with disease deterioration, such as low hemoglobin levels <10g/dl (p<0.02) and bone marrow infiltration >50% (p<0.053).



A



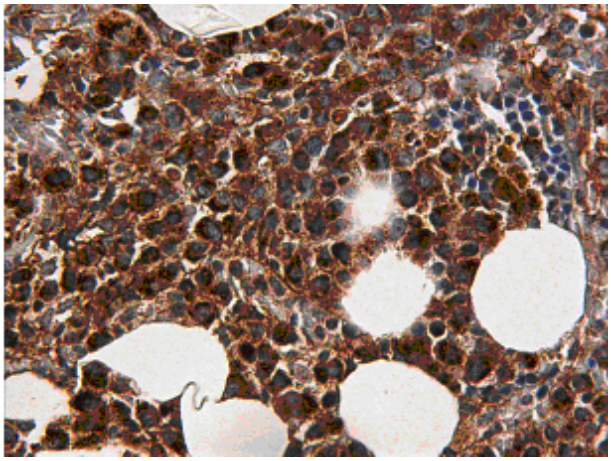
B

Figure 1. Trephine biopsy of two myeloma patients with high expression of PDGFRa **(A)** and intermediate expression **(B)** in plasma cells (x400).

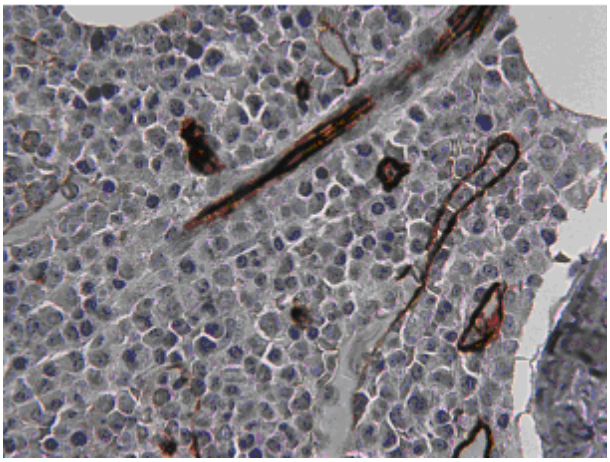
PDGFR β expression in bone marrow biopsies

The immunostaining for PDGFR β expression on PCs in the trephine biopsies revealed that 27 (15%) patients were negative (<20%) for PDGFR β expression (group I), while 27 (23%) showed intermediate positivity (group II) and 75 (62%) had a high PDGFR β positive expression on their

PCs (group III; Figure 2). The PDGFR β expression was correlated with several important parameters such as PFS and disease stage as per ISS. More precisely, it was shown that the patients who had high expression of PDGFR β in the PCs had unfavorable prognosis in terms of PFS (22 months median, range 18.14-25.86, p value 0.005, Figure 3). In addition PDGFR β high expression was correlated with advanced disease stage (ISS III, p<0.030) and was an independent factor in multivariate analysis.



A



B

Figure 2. Trephine biopsy of two myeloma patients with high expression of PDGFRβ **(A)** in plasma cells and intermediate grade of microvessel density **(B)**. (x400).

Expression of microvessel density on plasma cells in trephine biopsies

The bone marrow biopsies revealed that 30 (25%) patients had low grade (1-2) MVD (group I), while 19 (16%) showed intermediate grade (group II) and 71 (59%) had high grade (group III, Figure 2). MVD was not correlated with important parameters, such as PFS and disease stage as per ISS or the clinical response. In addition, MVD was not correlated neither with the PDGFR α nor with the PDGFR β.

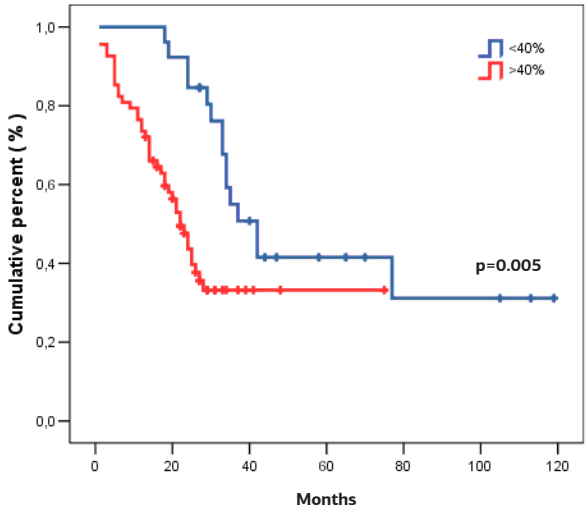


Figure 3. High expression of PDGFR β in the PCs shows unfavorable prognosis in terms of PFS.

Discussion

It is proved that angiogenesis plays a significant role in the pathogenesis of malignancies, both in solid or non solid tumors like MM. Several and diverse factors contribute in the development of angiogenesis and among them are the PDGFRs. Few studies have investigated the expression of the PDGFR α and PDGFR β in MM and have shown the correlation and the impact of these factors in the prognosis of MM patients [19]. The prognostic factors that have value in the assessment of NDMM patients could be classified into biochemical markers (LDH, haemoglobin, calcium, albumin, renal function, beta2 microglobulin) [20], imaging markers through specific techniques (MRI, PET-CT, whole body low dose CT) [11], genetic markers like rearrangement in the genetic material (deletion on chromosome 17, deletion on chromosome 13, abnormalities in 1q region, translocations t(4;14) and t(14;16)), to antigenic markers (CD38, CD56, CD27, MUM-1) and to pathology parameters like MVD [20]. In this study we assessed and analyzed the PDGFR α or CD140a, PDGFR β or CD140b and MVD in 120 NDMM patients. In our analysis there was not stratification based on the treatment and the administered therapeutic regimens that the patients had received. But we should mention that we may consider that all of the patients had a "uniform" treatment management as they were treated in the era of novel agents with proteasome inhibitor based regimens and immunomodulatory based regimens. Regarding the reported studies we have to underline the paper published by Coluccia et al. [21] based mainly on MM cell cultures and MM cell lines which revealed that dasatinib, a PDGFR β/Src

TKI, significantly delayed MM growth and angiogenesis *in vivo*. In addition, a paper published from Tsirakis et al. [15] indicated that there is a strong positive correlation between PDGF-AB and MVD and it seems that PDGF-AB plays a role in the complex network of cytokines inducing bone marrow neovascularization in patients with MM. They showed also that high MVD is significantly correlated with reduced survival time.

There is a continuous need for exploring and validating techniques and markers that can provide in a reproducible way directly and easily, information related to prognosis and the clinical impact on MM patients. In addition, the need for new therapeutic targets constantly exists. Our study attempted not to directly cover and fulfill but to explore and validate new techniques and biomarkers.

With the use of immunohistochemistry we explored the expression of two cell surface tyrosine kinase receptors the PDGFR α and β. The PDGFR family consists of PDGFR-a, -b, -c and -d, which form either homo- or heterodimers and are not active in their monomeric forms. The PDGFRs bind to the protein tyrosine kinase receptors PDGFR α and β. These two receptor isoforms dimerize upon binding the PDGF dimer. The dimerization is the prerequisite for the activation of the kinase. On the other hand the PDGFR may be present on different cell types via mitogen-activated protein kinases 8-10 (MAPK8-10) [22] and through a different pathway PDGFR activates directly the phosphoinositide-3-kinase (PI3K). The occurrence of clinically useful PDGFR antagonists, like imatinib mesylate, dasatinib, nilotinib, sorafenib, crenolanib, rinucumab, allows and "triggers" for the evaluation of the importance of the PDGFR in MM.

Our results confirm the expression of PDGFR α and β on PCs of NDMM patients and in addition indicate the correlation of PDGFR β with advanced disease. This study has limitations owing to its retrospective character and to the absence of stratification of the administered treatments.

In conclusion we observed high expression of PDGFR α and β in the PCs of NDMM patients. A positive correlation between high expression of PDGFR β and advanced disease was noticed. However, additional studies are needed for elucidating the importance of the PDGFRs as biomarkers in MM.

Conflict of interests

The authors declare no confict of interests.

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