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Expression of placental growth factor in bone marrow and peripheral blood of multiple myeloma patients

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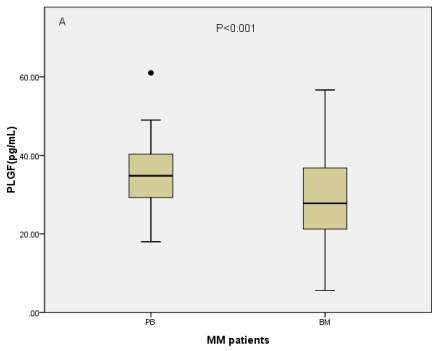
***Abstract*: Multiple studies have shown that neovascularization is closely associated with hematological malignancies. As a member of the vascular endothelial growth factor (VEGF) family, Placental Growth Factor (PLGF) play an important role in angiogenesis, progression, and prognosis of a variety of cancers. We determined the bone marrow (BM) and peripheral blood (PB) levels of PLGF in 37 multiple myeloma (MM) patients (18 were newly diagnosed and 19 were relapsed for further treatment) and 30 controls matched for age, sex, and chronic disease (such as cardiovascular disease and chronic obstructive pulmonary disease). Differential expression among different groups and associations with clinical and laboratory variables were conducted. We found the PLGF expression in the treatment naïve group was significantly higher than in the control group both in BM and PB (p < 0. 05). Compared with the treatment naïve group, PLGF was significantly lower in the retreatment group (p < 0. 05). PLGF was significantly higher in stage III compared to stages I and II (P <0.05). PB-PLGF was significantly correlated with serum β 2-microglobulin and albumin levels and PLGF between BM and PB was significantly correlated. We conclude that elevated PLGF play an important role in the growth, and progression of MM and associated with prognosis.**



Keywords: Multiple myeloma; Placental growth factor; Prognosis

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1. Introduction

Angiogenesis is closely related to development and prognosis of malignant hematological tumors such as lymphoma [1], acute Leukemia [2], myelodysplastic syndrome [3] and multiple myeloma [4]. Placental growth factor (PLGF) was initially isolated and purified from human placenta cDNA library as a new angiogenic growth factor by Maglione in 1991 [5] and belongs to the family member of vascular endothelial growth factor (VEGF), which is a pleiotropic cytokine that can stimulate endothelial cells growth, migration, survival and stimulation of angiogenesis by binding to VEGF receptor 1 (also known as FLT1) [6]. Clinical observations and studies have shown that the overexpressed PLGF mRNA or protein is positively associated with pathological angiogenesis, tumor cell growth, invasion, advanced tumor stage, recurrence and poor prognosis of multiple cancers [6-8].

Multiple myeloma (MM) is a common haematological neoplasm originated from pre-switched, follicle center B-lymphocytes, which differentiate to malignant clonal plasma cells in bone marrow (BM). Clinical manifestations include pathologic fractures, anemia, hypercalcemia and renal dysfunction. It accounts for more than 10% of all hematological malignancies. Several studies demonstrated that bone marrow microvessel density in MM patient was increased and associated with the tumor growth and patient prognosis [4, 9-11]. Multiple cytokines and PLGF are suggested with involvement in angiogenesis and carcinogenesis [6]. Although studies have shown that PLGF is related to the occurrence, development and prognosis of a variety of tumors, but without knows its role in MM.

In this study, we measured the expression levels of PLGF in both bone marrow and peripheral blood of the MM patients and controls and evaluated the association of PLGF expression with clinical variables and their potential clinical implications.

2. Materials and methods

2.1. Patients

Thirty seven cases of MM patients and thirty medical-history (which variables were matched, age, gender, chronic disease) –matched controls were enrolled to the study. Among the MM patients, (18 were newly diagnosed before treatment and 19 were being treated with Bortezomib/dexamethasone (BD), Vincristine/doxorubincin/ dexamethasone (VAD) or Dexamethasone/cyclophosphamide/etoposide/cisplatin (DECP) regimens. The diagnoses were established in the Department of Hematology, Affiliated Hospital of Qingdao University during the period of December 2013 to September 2014, according to the International Myeloma Working Group Criteria [12]. Patient clinical stages were made staged according to the classification of Durie-Salmon [13]. Clinical manifestations associated with outcomes of MM were defined as:

anemia, hemoglobin below the lower normal limit over 20 g/L, 138 g/L for men and 119 g/L for women; renal dysfunction, serum creatinine level above the upper limits of normal (117μmol/L for men and 96μmol/L for women); and bone disease, any of osteolytic lesions or severe osteoporosis with compression fractures on standard X-ray of the bones. Additionally, these clinical outcomes indicators were measured: β2-microglobulin (normal values less than 2.5mg/L), serum free light chains (normal range between 3-19mg/L), and serum albumin (normal range between 30-50g/L). The controls consisted of thirty volunteers who were clinic patients with abnormal hemogram but none were determined with any hematological disease or other malignant tumors. Because studies showed that PLGF expression was elevated some common geriatric diseases such as cardiovascular disease [14-15] and chronic obstructive pulmonary disease [16], the volunteers with the same medical historyals the MM patients were selected. Written informed consent was obtained for each patient and healthy volunteer. Our study and the collection of all human samples were approved by the Institutional Review Board of the University Hospitals of Qingdao and written informed consent was obtained from all patients.

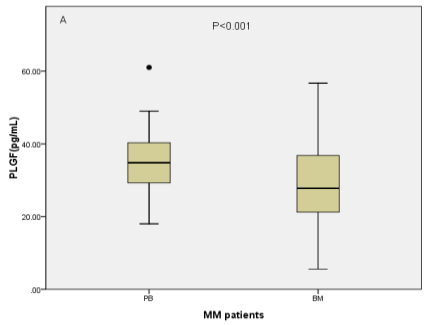

Figure 1. The differences levels of PLGF between PB and BM both in MM patients (paired t test).

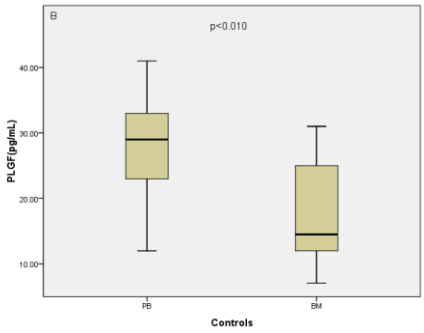

Figure 2. The differences levels of PLGF between PB and BM both in controls (B) (paired t test).

2.2. Sample collection

BM and PB samples were collected at newly diagnosed or during the treatment under sterile conditions. Each sample was centrifuged (2000xg) within-ten mins after collection at low temperature, the supernatant was transferred into the EP tubes, and stored at -80 ℃ until assayed. The concentration of the PLGF in the supernatant was detected by ELISA assay according to manufacturer's instructions for PLGF.

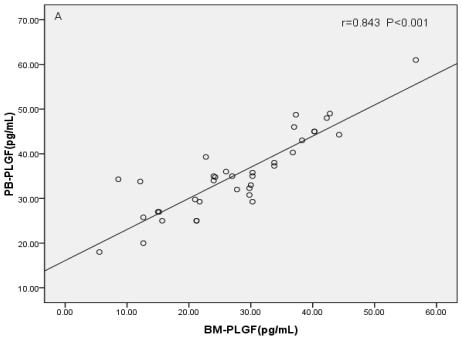

Figure 3. Correlation of PB-PLGF with BM-PLGF.

2.3. Statistical analysis

SPSS17.0 software was used for statistical analysis. Means± SD was used for continuous variables. Two sample independent t test, one-way analysis of variance (ANOVA) or the Mann–Whitney U test (nonparametric alternative) was performed for group differences. The Spearman's rank correlation coefficient was used to determine the correlation among clinical indicators and PLGF. Between each patient's bone marrow and peripheral blood, paired t test was applied. P <0.05 was considered as statistical significance.

3. Results

3.1. Clinical characteristics of study patients and controls

The patient characteristics are summarized in Table 1. The 22 men and 15 women are with median age 61 years, ranging from 41 to 89 years. The controls are with a median age of 59 years, ranging from 35 to 72 years. Where 1 patient had stage I, 10 patients had stage II, and 26 patients had stage Ⅲ disease. Since the number of cases is less in each stage groups, we grouped clinical stages Ⅰ and Ⅱ together and compared them to stage III which with the largest tumor mass.

3.2. PLGF expression in BM and PM between MM patents and controls

The mean and standard deviation of PLGF expression in the MM patents and controls are presented in Table 2.The treatment naïve group's PLGF expression levels were significantly higher in both BM and PB than in the control group. The retreatment group had significant lower expression of PLGF compared with the treatment naïve group (P <0.05). No significant difference was observed for the

rest of comparisons (P >0.05). Moreover, significant differences were observed for the PLGF expression between PB and BM, both in MM patients (Fig 1) and controls (Fig 2). And, PB-PLGF and BM-PLGF expressions were highly positively correlated (r=0.843, p<0.001) (Fig 3).

Table 1 Clinical features of the MM patents and controls

Clinical features	Patents with MM (N=37)	Controls (N=30)
Sex		
Male	22	20
Female	15	10
Age		
≤65	21	17
>65	16	13
Medical history		
Health	5	6
Unhealthy [a]	19	21
Durie-Salmon stage		
Ⅰ/Ⅱ	11	
Ⅲ	26	
Renal dysfunction [b]		
Yes	12	
No	25	
bone disease [c]		
Yes	24	
No	13	
Anemia [d]		
Yes	33	
No	4	
β2-microglobulin	Median(range)	
(≤2.7mg/L) [e]	4.68(1.5-11.3)	
Serum albumin	Median (range)	
(30–50 g/liter) [e]	34.78(17.04-44.8)	
Serum free light chains [e]	Median (range) 7.28(2.23-88.25)	

Note: [a]patients with medical history like cardiovascular disease and chronic obstructive pulmonary disease; [b]serum creatinine level above the upper limit of normal; [c]any of osteolytic lesions or severe osteoporosis with compression fractures on standard X-ray of the bones; [d]hemoglobin value below the lower limit of normal 20 g/L; [e]Characteristic (normal range).

3.3. Association of PLGF expression with clinical characteristics of MM Patients

No significant difference of PLGF expression was observed between the patients in different age groups, different genders, and with or without renal insufficiency, bone disease and anemia in both serum and bone marrow (Table 3). However, when comparing the PLGF expression in PB and BM with regards to tumor burden, we observed that the stage III patients had significantly elevated PLGF compared to stages I and II patients in both serum and bone marrow (P<0.05). Additionally, we analyzed the level of serum β2-microglobulin, serum free light chains and serum albumin between the treatment naïve patients and the retreatment patients and found that β2-microglobulin was significantly higher and serum albumin was significantly lower in the treatment naïve group than the retreatment group (Table 3). We also found that PB-PLGF was in positive correlation with the serum β2-microglobulin(r=0.402, p<0.028)(Fig 4) and negative with the serum albumin (r=-0.483, p<0.007) (Fig 5).

4. Discussion

With bortezomib and thalidomide's application and high-dose chemotherapy supported by PB stem cell transplantation, the treatment of MM has made great progress with significantly improved complete remission rate and overall survival in recent years. However MM still is an incurable disease. Increasing evidence suggests that increased angiogenesis in the bone marrow is a strong indication of an aggressive disease and a poor prognosis as seen in other hematological malignancies and solid tumors. A variety of cytokines seems influencing angiogenesis by mediating the autocrine and paracrine relationships between tumor cells and stroma, which includes VEGF, interleukin-6, G protein-coupled receptor 124, basic fibroblastic growth factor and insulin-like growth-factor-1[16-20]. Ongoing research continues to unravel the interactions between these players in the pathogenesis and progression of MM. As a member of the VEGF family, PLGF can cause leukocyte infiltration, tumor growth, stromal cell migration, ischemic tissue revascularization [6].The over expression of PLGF correlates with advanced progression and poor prognosis of multiple tumors, like non-small cell lung cancer, colorectal cancer, and stomach cancer [21-23]. However, there is no answer to the role and the possible clinical significance of PLGF in MM.

In this study, we demonstrated increased PLGF expression in untreated MM patients compared with the controls as well as with the patients previously treated, which raised a possible role of PLGF in the growth of MM. We also found that the concentrations of PLGF both in BM and PB were significantly elevated in stage III compared with stages I and II patients of Durie-Salmon clinical stage. This positive association suggests PLGF value is likely related to tumor burden.So, the level of PLGF which produced by malignant plasma cells might represent a measure which based on a mathematical model for estimating the number of tumor cells rather than the Durie-Salmon Staging System. Indeed, multiple

studies found that high expression of PLGF mRNA or protein is significantly associated with an advanced clinical stage for like gastric, lung, and colorectal cancers.

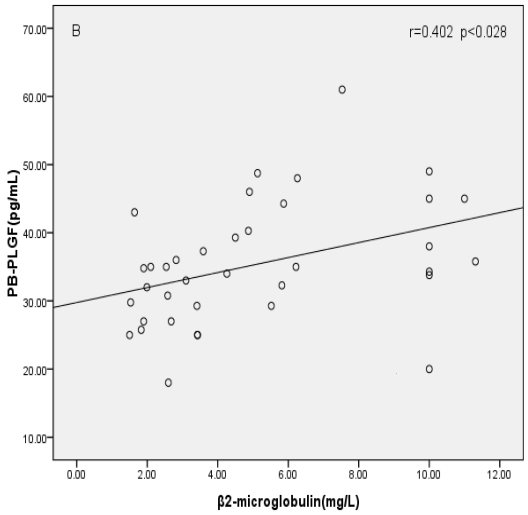

Figure 4. Correlation of β2-microglobulin.

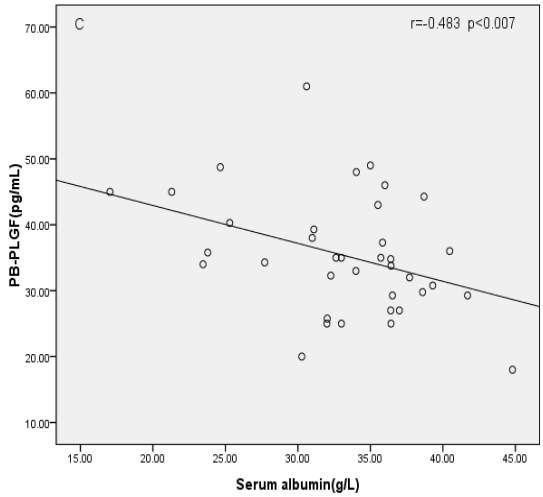

Figure 5. Correlation of Serum albumin.

As we all know, MM is a collection of related disorders rather than a single disease. Thus, the present study analyzed the correlation between PLGF expression level and the most common presenting symptoms of MM (e.g., anemia, renal insufficiency, and bone disease). Our study revealed that neither plasma nor bone marrow PLGF level was associated with these symptoms of MM, which suggests that this cytokine may not be relevantly involved in these clinical presentations.

A certain body of evidence demonstrated that β2-microglobulin [24], serum free light chains [25] and albumin [26] were predictive for response and survival. Consistent with the findings, our data showed the naïve patients had significant higher concentrations of β2-microglobulin, as well as lower albumin. Both indicators were associated with PB-PLGF levels. All these suggest that a high PB-PLGF concentration is associated with unfavorable clinical outcomes. Although the same associations were not seen in bone marrow it may be due to the relatively small number of cases included in this study.

Our study also found that serum level of PLGF is highly correlated with bone marrow level and is also expressed at higher level. Studies have shown that the serum level of PLGF may be elevated in some common diseases like cardiovascular disease, chronic obstructive pulmonary disease.

In recent years, both pre-clinical and clinical studies has not come up with a unified theory or mechanism to describe the role of PLGF as a factor involved in the promotion or inhibition of tumor angiogenesis and growth[27]. Nevertheless, a number of studies have shown that anti-PLGF antibodies could inhibit tumor growth and metastasis in ectopic and orthotopic model [28-30]. Withing the anti-angiogenesis therapy has become a hot topic in tumor targeted therapy, PLGF provides a new therapy target for cancer therapy. Studies have shown that anti-angiogenic therapy with lenalidomide could have marked activity in MM[31]. At present, there is no cure for MM, and new therapeutic targets are still under exploration. The role of PLGF in the MM still need further investigation. However, this study provides new therapeutic target for the treatment of anti-MM.

Table 2 mean PLGF expression levels in the MM patents and controls (mean ±SD;pg/ml)

Material	PLGF			F
	Treatment naïve (N=18)	Retreatment (N=19)	Controls (N=30)	
PB	39.23±9.98*#	31.71±6.94	26.50±8.78	0.008
BM	32.31±12.21*#	23.31±9.49	16.76±8.51	0.010

Note: *P<0.05 in comparison to the control group in ANOVA; # P<0.05 in comparison to the retreatment group in ANOVA.

Table 3 The correlation between PLGF expression and the clinical pathological factors (mean ±SD;pg/ml)

Variables	Characteristic	PB	P	BM	P
PLGF	Sex				
	Male	35.58±10.47	0.866	27.41±13.18	0.658*
	Female	35.01±7.34		28.08±9.30	
	Age				
	≤65	34.97±7.91	0.788	28.39±8.54	0.623*
	>65	35.87±11.09		26.71±15.21	
	Durie-Salmonstage				
	Ⅰ/Ⅱ	30.57±8.29	0.047	21.04±10.29	0.038
	Ⅲ	37.68±8.91		30.55±11.42	
	Renal dysfunction				
	Yes	37.22±6.83	0.299	31.46±9.17	0.106
	No	34.25±7.26		25.07±9.94	
	Bone disease				
	Yes	34.34±6.673	0.306	28.25±9.03	0.498
	No	37.35±7.85		25.45±12.15	
	Anemia				
	Yes	36.35±9.22	0.238*	29.01±11.76	0.132*
	No	29.76±7.95		20.21±8.37	
	Medical history				
	Health	37.31±10.63	0.859*	28.54±13.43	0.972*
	Unhealthy	37.65±8.97		29.32±12.25	
β_2-microglobulin	Groups				
	Treatment naïve	6.90±3.20	0.006*		
	Retreatment	3.69±2.27			
Serum albumin	Groups				
	Treatment naïve	29.81±6.33	0.011		
	Retreatment	35.63±5.47			
Serum free light chains	Groups				
	Treatment naïve	19.46±23.18	0.120*		
	Treatment	9.58±9.17			

Note: * tested using Mann-Whitney test when the data is normally distributed or variance missing, without the *using independent samples t test.

5. Conclusion

Our results indicate that increased serum and bone marrow PLGF in untreated MM patients as well as in patients with advanced MM stage. The expression levels can be reduced after treatment. Serum PLGF was significantly correlated with β2-microglobulin and albumin. The observations support the notion that PLGF play an important role in the growth and progression of MM and patient prognosis. PLGF may be a potential therapeutic target in anti-MM therapy.

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References

[1] Marinaccio C, Nico B, Maiorano E, Specchia G, Ribatti D. Insights in Hodgkin Lymphoma angiogenesis. Leuk Res, 38(8): 2014 857-861.

[2] Haouas H. Angiogenesis and acute myeloid leukemia. Hematology, 19(6): 2014 311-323.

[3] Savic A, Cemerikic-Martinovic V, Dovat S, Rajic N, Urosevic I, Sekulic B, Kvrgic V, Popovic S. Angiogenesis and survival in patients with myelodysplastic syndrome. Pathol Oncol Res, 18(3): 2012 681-690.

[4] Vacca A, Ria R, Reale A, Ribatti D. Angiogenesis in multiple myeloma. Chem Immunol Allergy, 99: 2014 180-196.

[5] Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG. Isolation of human placenta cDNA acording for a protein related to the vascular permeability factor. Proc Natl Acad Sci USA, 88(20): 1991 9267–9271.

[6] Fischer C, Mazzone M, Jonckx B, Carmeliet P. FLT1 and its ligands VEGFB and PLGF: drug targets for anti-angiogenic therapy. Nat Rev

Cancer, 8(12): 2008 942-956.
[7] Marcellini M, De Luca N, Riccioni T, Ciucci A, Orecchia A, Lacal PM, Ruffini F, Pesce M, Cianfarani F, Zambruno G, Orlandi A, Failla CM. Increased melanoma growth and metastasis spreading in mice overexpressing placenta growth factor. Am J Pathol, 169(2): 2006 643-654.
[8] Parr C, Watkins G, Boulton M, Cai J, Jiang WG. Placenta growth factor is over-expressed and has prognostic value in human breast cancer. Eur J Cancer, 41(18): 2005 2819-2827.
[9] Sucak GT, Aki SZ, Yüzbaşioğlu B, Akyürek N, Yağci M, Bağriaçik U, Haznedar R. Prognostic value of bone marrow microvessel density and angiogenic cytokines in patients with multiple myeloma undergoing autologous stem cell transplant. Leuk lymphoma, 52(7): 2011 1281-1289.
[10] Bhatti SS, Kumar L, Dinda AK, Dawar R. Prognostic value of bone marrow angiogenesis in multiple myeloma: use of light microscopy as well as computerized image analyzer in the assessment of microvessel density and total vascular area in multiple myeloma and its correlation with various clinical, histological, and laboratory parameters. Am J Hematol, 81(9): 2006 649-656.
[11] International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. Br J Haematol, 121(5): 2003 749–757.
[12] Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer, 36(3): 1875 842–854.
[13] Roncal C, Buysschaert I, Gerdes N, Georgiadou M, Ovchinnikova O, Fischer C, Stassen JM, Moons L, Collen D, De Bock K, Hansson GK, Carmeliet P. Short-term delivery of anti-PLGF antibody delays progression of atherosclerotic plaques to vulnerable lesions. Cardiovasc Res, 86(1): 2010 29-36.
[14] Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, Liao F, Nagy JA, Hooper A, Priller J, De Klerck B, Compernolle V, Daci E, Bohlen P, Dewerchin M,Herbert JM, Fava R, Matthys P, Carmeliet G, Collen D, Dvorak HF, Hicklin DJ, Carmeliet P. Revascularization of ischemic tissues by PLGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis byanti-Flt1. Nat Med, 8(8): 2002 831-840.
[15] Cheng SL, Wang HC, Yu CJ, Yang PC. Increased expression of placenta growth factor in COPD.Thorax, 63(6):2008 500-506.
[16] Gianni-Barrera R, Bartolomeo M, Vollmar B, Djonov V, Banfi A.Split for the cure: VEGF, PDGF-BB and intussusception in therapeutic angiogenesis. Biochem Soc Trans, 42(6): 2014 1637-1642.
[17] Nagasaki T, Hara M, Nakanishi H, Takahashi H, Sato M, Takeyama H.Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour-stroma interaction. Br J Cancer, 110(2): 2014 469-478.
[18] Wang Y, Cho SG, Wu X, Siwko S, Liu M.G-protein coupled receptor 124 (GPR124) in endothelial cells regulates vascular endothelial growth factor (VEGF)-induced tumor angiogenesis.Curr Mol Med, 14(4): 2014 543-554.
[19] Wilkins JR, Pike DB, Gibson CC, Kubota A, Shiu YT.Differential effects of cyclic stretch on bFGF- and VEGF-induced sprouting angiogenesis. Biotechnol Prog, 30(4): 2014 879-888.
[20] Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M,Hideshima T, Chauhan D, Joseph M, Libermann TA, García-Echeverría C,Pearson MA, Hofmann F, Anderson KC, Kung AL. Inhibition of the insulin-like growth factor receptor-1tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. Cancer Cell, 5(3): 2004 221-230.
[21] Zhang L, Chen J, Ke Y, Mansel RE, Jiang WG. Expression of Placenta growth factor (PLGF) in non-small cell lung cancer (NSCLC) and the clinical and prognostic significance. World J Surg Oncol, 3: 2005 68-79.
[22] Sung CY, Son MW, Ahn TS, Jung DJ, Lee MS, Baek MJ.Expression of placenta growth factor in colorectal carcinomas. J Korean Soc Coloproctol, 28(6): 2012 315-320.
[23] Chen CN, Hsieh FJ, Cheng YM, Cheng WF, Su YN, Chang KJ, Lee PH. The significance of placenta growth factor in angiogenesis and clinical outcome of human gastric cancer. Cancer Lett, 213(1): 2004 73-82.
[24] Rossi D, Fangazio M, De Paoli L, Puma A, Riccomagno P, Pinto V, Zigrossi P, Ramponi A, Monga G, Gaidano G. Beta-2-microglobulin is an independent predictor of progression in asymptomatic multiple myeloma. Cancer, 116(9): 2010 2188-2200.
[25] Larsen JT, Kumar SK, Dispenzieri A, Kyle RA, Katzmann JA, Rajkumar SV. Serum free light chain ratio as a biomarker for high-risk smoldering multiple myeloma. Leukemia, 27(4): 2013 941-946.

[26] Kim JE, Yoo C, Lee DH, Kim SW, Lee JS, Suh C. Serum albumin level is a significant prognostic factor reflecting disease severity in symptomatic multiple myeloma. Ann Hematol, 89(4): 2010 391-397.
[27] Domenico Ribatti. The controversial role of placental growth factor in tumor growth. Cancer Letters, 307(1): 2011 1-5.
[28] Cursiefen C, Chen L, Borges LP, Jackson D, Cao J, Radziejewski C, D'Amore PA, Dana MR, Wiegand SJ, Streilein JW. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Invest, 113(7): 2004 1040-1050.
[29] Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, Squadrito ML, Segura I, Li X, Knevels E, Costa S, Vinckier S, Dresselaer T, Åkerud P, De Mol M, Salomäki H, Phillipson M, Wyns S, Larsson E, Buysschaert I, Botling J, Himmelreich U, Van Ginderachter JA, De Palma M, Dewerchin M, Claesson-Welsh L,Carmeliet P. HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through down regulation of PLGF. Cancer Cell, 19(1): 2011 31-44.
[30] Martinsson-Niskanen T, Riisbro R, Larsson L,Winstedt L, Stenberg Y, Pakola S, Stassen JM, Glazer S. Monoclonal antibody TB-403: a first-in-human, phase I, double-blind, dose escalation study directed against placental growth factor in healthy male subjects.Clin Ther, 33(9): 2011 1142-1149.
[31] Andhavarapu S, Roy V. Immunomodulatory drugs in multiple myeloma.Expert Rev Hematol, 6(1): 2013 69-82.