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RESEARCH

Interleukin-10 Induces Both Plasma Cell Proliferation and Angiogenesis in Multiple Myeloma

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Abstract In multiple myeloma the angiogenic process is enhanced by various mediators. Among them interleukin-10 (IL-10), secreted mainly by myeloma-associated macrophages seems to participate in myeloma progression with variable manners. The aim of the study was to measure serum levels of IL-10 in various stages of MM patients and to correlate them with various angiogenic cytokines, such as vascular endothelial growth factor and angiopoietin-2 and with known proliferation parameters, such as serum levels of B-cell activating factor and bone marrow infiltration by myeloma plasma cells, in order to explore their clinical significance. We measured serum levels of the above parameters by ELISA in 54 newly diagnosed MM patients. All of them were higher in MM patients and were increasing in parallel with disease progression. Furthermore, IL-10 correlated positively with both angiogenic cytokines and proliferation markers. This correlation of IL-10 with both angiogenic cytokines and markers of disease activity implicates that they all have an important role in MM pathogenesis and progression.

Keywords Angiogenesis · Cytokines · Growth factors · Multiple myeloma

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Introduction

Multiple myeloma (MM) is a clonal B-cell neoplasia characterized by the accumulation of malignant plasma cells within the bone marrow (BM), in close contact with other cells of its microenvironment. MM cells and BM microenvironment activate the autocrine or paracrine secretion of various mediators. Among them, angiogenic factors promote tumor cell growth and survival, a required step for the progression of tumor growth, invasion and metastasis. MM-induced angiogenesis is a complex process, with the participation of various regulators. Its mechanisms include the production of cytokines by myeloma cells and the induction of their secretion by microenvironmental cells. Stromal cells support MM cell growth and survival [1]. Among them, inflammatory cells, such as macrophages, play an important role in tumor growth, angiogenesis and disease progression [2, 3].

Interleukin-10 (IL-10) is a cytokine produced by monocytes/macrophages, T and B lymphocytes, NK cells, as well by mast cells [3]. IL-10 can significantly enhance the proliferation of B cells, being involved in their terminal differentiation into plasma cells [4]. IL-10 has been implicated in the pathogenesis of other malignant B-cell neoplasms [5, 6], such as chronic lymphocytic leukemia and diffuse large B-cell lymphoma, where its serum levels in untreated patients are increased and have been correlated with an inferior survival [7]. Thus, IL-10 may be involved at different steps of B lineage expansion and differentiation. Furthermore, it seems to act as a growth factor for MM cells [8]. Increased serum levels of IL-10 have been associated with advanced MM stage [9].

Malignant plasma cells can secrete vascular endothelial growth factor (VEGF), a well-known pro-angiogenic cytokine, in response to interleukin-6 (IL-6) stimulation; in turn, microvascular endothelial and BM stromal cells secrete IL-6, a potent growth factor for malignant plasma cells, in response to VEGF stimulation [10, 11].

Maturation and stabilization of the vascular wall are critically regulated by angiopoietin-1 (Ang-1), which binds primarily to Tie-2 receptor, expressed by endothelium [12]. By contrast, angiopoietin-2 (Ang-2) antagonizes Tie-2 binding and induces vessel destabilization, leading to the angiogenic sprouting [13]. The expression of angiopoietins has been investigated in human solid tumors, including cancer of breast, liver, lung, thyroid, colon and stomach [14, 15]. These studies show an important role of Ang-2, in cooperation with VEGF, in the initiation of tumoral angiogenesis. The expression of Ang-2 by endothelial cells has also been observed in isolated BM endothelial cells from patients with MM [16].

B cell-activating factor (BAFF), a member of tumor necrosis factor (TNF) super-family, is critical for the maintenance of normal B-cell development and homeostasis. It is also a myeloma cell growth factor. Myeloma cell lines and primary myeloma cells express BAFF and its receptors. Furthermore, high serum BAFF levels have been detected in newly diagnosed MM patients, being correlated with disease stage and known factors of disease activity and decreased after effective treatment [17].

The aim of the study was to measure serum levels of IL-10 in MM patients with varying severity of disease and to correlate them with various angiogenic cytokines, such as VEGF and Ang-2 and with proliferation parameters, such as BAFF and BM infiltration, in order to explore their clinical significance.

Patients and Methods

Patients

Fifty-four newly diagnosed MM patients (28 males, 26 females) were included in this study. Their median age was 69 years (range 49–85 years). According to international staging system (ISS) 15 were classified as stage I, 20 as stage II and 19 as stage III. Concerning the types of monoclonal protein, 30 had IgG, 18 IgA and 6 light chain disease. Twenty, age- and sex–matched healthy subjects were used as controls. The work has been carried out in accordance with the Declaration of Helsinki. This study has been cleared by our Institution Ethics Review Board for human studies and all subjects have signed an informed consent.

Methods

Serum samples, from patients, before starting any myeloma-related therapy, and controls, were collected, stored at −70 ° C and analyzed at the end of the study, in order to avoid inter-assay variability. The detection of BAFF, Ang-2, VEGF and IL-10 in the serum was performed by a solid-phase sandwich ELISA, using monoclonal antibodies against BAFF, Ang-2, VEGF and IL-10 (Quantikine, R & D systems Inc. Mineapolis, MN, USA) according to the manufacturers' instructions.

Transiliac BM samples were collected from MM patients at diagnosis. The pattern of BM plasma cell infiltration was highlighted by their immunostaining with a monoclonal antibody to CD38. Monoclonality and percentages of κ/λ neoplastic cells in the BM were assessed by in situ hybridisation.

Statistical Analysis

Results are expressed as mean±SD. The non-parametric Mann–Whitney test was applied to assess possible differences between patients and controls. The non-parametric Kruskal-Wallis test and one-way analysis of variance (ANOVA) were assessed to examine the existence of differences between different stages. Correlations between the various measured parameters were calculated by the Spearman's rank correlation coefficient. A 5 % significance level was applied.

Results

All measured parameters were significantly higher in newly diagnosed patients compared to healthy population (Table 1). Furthermore, they were also increasing in parallel with disease progression, with higher values in advanced ISS stages (Table 2).

Serum levels of VEGF correlated positively with BAFF (r=0.356), IL-10 (r=0.579) (Fig. 1), infiltration (r=0.641) and Ang-2 (r=0.729) (p=0.008 for BAFF and p<0.0001 for the other cases). Similarly, Ang-2 correlated significantly with BAFF (r=0.547), IL-10 (r=0.487) (Fig. 2) and infiltration (r=0.549) (p<0.0001 for all cases). Significant correlations between BAFF with IL-10 (r=0.397, p<0.003) (Fig. 3) and infiltration (r=0.387 p<0.004) and between IL-10 with infiltration (r=0.461 p<0.0001) (Fig. 4) were also found.

Discussion

The cellular and molecular basis of MM progression is favored by mechanisms involving the BM microenvironment. Angiogenesis represents a main feature of disease progression and is supported by all cellular and extracellular elements of

Table 1 Mean±SD values of angiopoietin-2 (Ang-2), vascular endothelial growth factor (VEGF), interleukin-10 (IL-10) and B cell activating factor (BAFF) in multiple myeloma patients and in healthy controls

	ANG-2 (pg/ml)	VEGF (pg/ml)	IL-10 (pg/ml)	BAFF (pg/ml)
Controls	277.6±49.5	87.3±18.7	103.7±16.1	252.7±185.4
Patients	660±449.3	259.2±168.3	185.5±79.0	880.1±552.0
p-value	<0.001	<0.001	<0.001	<0.001

Table 2 Mean±SD values of angiopoietin-2 (Ang-2), vascular endothelial growth factor (VEGF), interleukin-10 (IL-10), B cell activating factor (BAFF) and bone marrow infiltration from plasma cells, between myeloma stages (ANOVA test)

	ANG-2 (pg/ml)	VEGF (pg/ml)	IL-10 (pg/ml)	BAFF (pg/ml)	Infiltration (%)
Stage 1	268.8±100.6	139.2±74.9	140.3±29.4	537.6±224.7	23.5±10.3
Stage 2	577.3±309.5	252.8±165.9	173.6±63.4	901.2±497.0	39.0±12.0
Stage 3	1056.1±429.7	383.9±148.8	233.6±95.7	1130.2±658.0	56.1±15.9
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

BM niche. It is uncontrolled, unlimited in time and essential for tumor growth, invasion and metastasis during the transition from the avascular to the vascular phase [18].

Macrophages play an important role in a wide variety of biological functions, such as neovascularization [19]. In oncology, tumor-associated macrophages (TAMs) coordinate various aspects of the malignancy, such as tumor growth and progression, angiogenesis, metastasis, immunosuppression, and tissue remodeling. In tumor microenvironment various molecules act as chemoattractants for macrophages, such as IL-4, IL-13, transforming growth factor-beta (TGF-beta), and IL-10 [20]. After stimulation, TAMs may produce high levels of IL-10. This autocrine and paracrine network of IL-10 stimulation seems to possess a significant activating role on the macrophages, promoting the production of IL-4 and IL-13 by Th2 cells [21], and consequently participating in tissue remodeling, immune modulation, and tumor progression [22]. Furthermore, TAMs are able to modulate, induce and regulate neovascularization [23]. Since TAMs are accumulated in hypoxic areas of tumors, when they get activated they acquire the ability to secrete various pro-angiogenic mediators (including growth factors, cytokines, proteases, and chemokines) in order to promote angiogenesis and to influence tumor growth [24]. By these means, TAMs release growth factors, such as VEGF, platelet-derived growth factor, TGF-beta, and a member of the fibroblast growth factor family, and proteases, such as matrix metalloproteases, plasmin, and urokinase plasminogen, which all participate on angiogenesis [25–27]. It seems that IL-10 and hypoxia have a synergistic role to influence macrophage function and angiogenic homeostasis, suggesting that the pro-angiogenic effects of hypoxic macrophages are IL-10-dependent and require IL-10 mediated signal transduction [23].

IL-10 is an important cytokine in tumor microenvironment, being expressed by TAMs, tumor and CD8+ T-cells. IL-10 can be regarded as an anti-inflammatory and immunosuppressive cytokine, favoring tumor escape from immune surveillance. The autocrine circuit of TAM-derived IL-10 may suppress the expression of the potential antitumor IL-12 [28]. Nevertheless, immunosupressive effect of IL-10 is not steady, since it has been postulated to possess some immunostimulating properties, playing, by these means, important role in antitumor response [29–31]. All the above information suggests the controversial role of IL-10 in tumor microenvironment.

In the present study we confirmed higher serum levels of IL-10 in MM patients compared to controls and moreover, increased in advanced disease [9]. We also confirmed

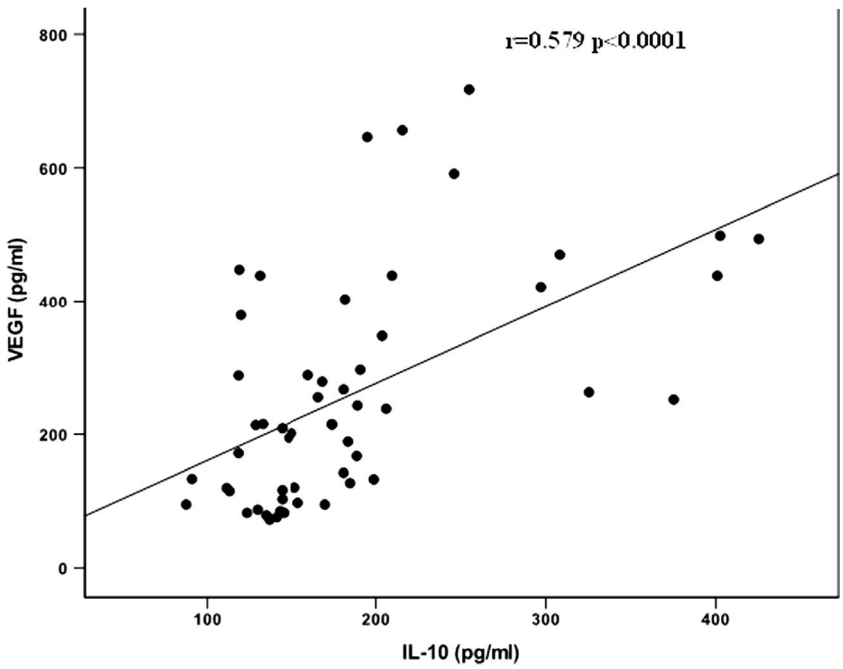

Fig 1 Positive correlation between serum levels of interleukin-10 (IL-10) with vascular endothelial growth factor (VEGF), in multiple myeloma patients

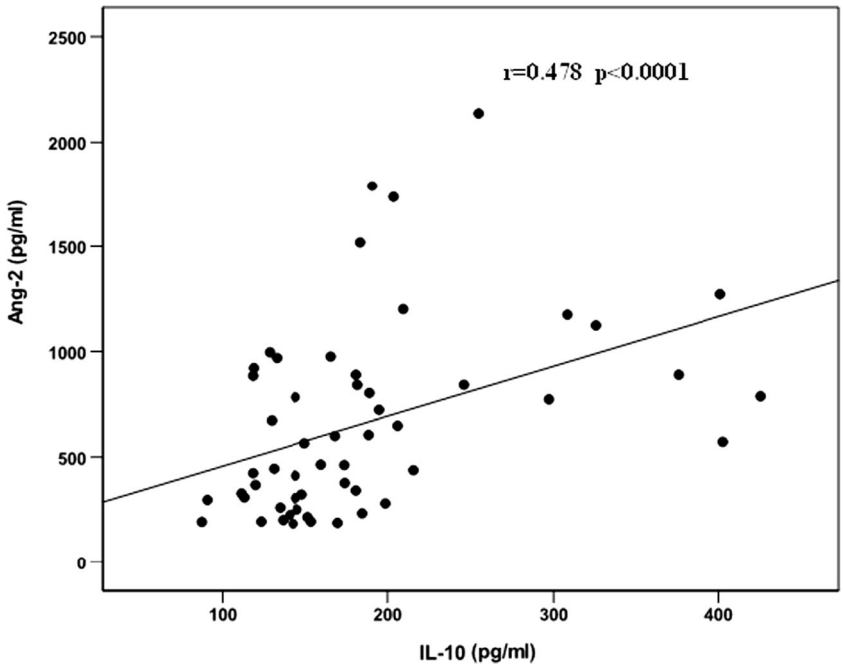

Fig 2 Positive correlation between serum levels of interleukin-10 (IL-10) with angiopoietin-2 (Ang-2), in multiple myeloma patients

significant correlations with markers of proliferation (BAFF serum levels and bone marrow infiltration), as have been correlated with levels of IL-6, Il-15, C-reactive protein (CRP) and beta-2 microglobulin [9]. Moreover, we found correlations with markers of angiogenesis (VEGF and Ang-2 serum levels). BAFF serum levels have been correlated with serum levels of IL-6, lactic dehydrogenase and CRP, being all well known markers of disease activity [17]. In a series of MM cell lines, it was reported that IL-6 leads to a marked production of IL-10, suggesting that it is an IL-6- related growth factor for MM cells [8]. Consequently, elevated IL-10 levels in MM patients can be related to the clinical manifestation and stage of the disease.

In MM, macrophages possess an abundant and important component of stromal cells, and their infiltration has been associated with vascularity and prognosis [32]. In active MM, plasma cells secrete angiogenic cytokines such as VEGF and FGF-2 inducing inflammatory cells to secrete their own angiogenic factors. All these cytokines recruit and activate MM-associated macrophages to collaborate with other cells in vessel formation [33].

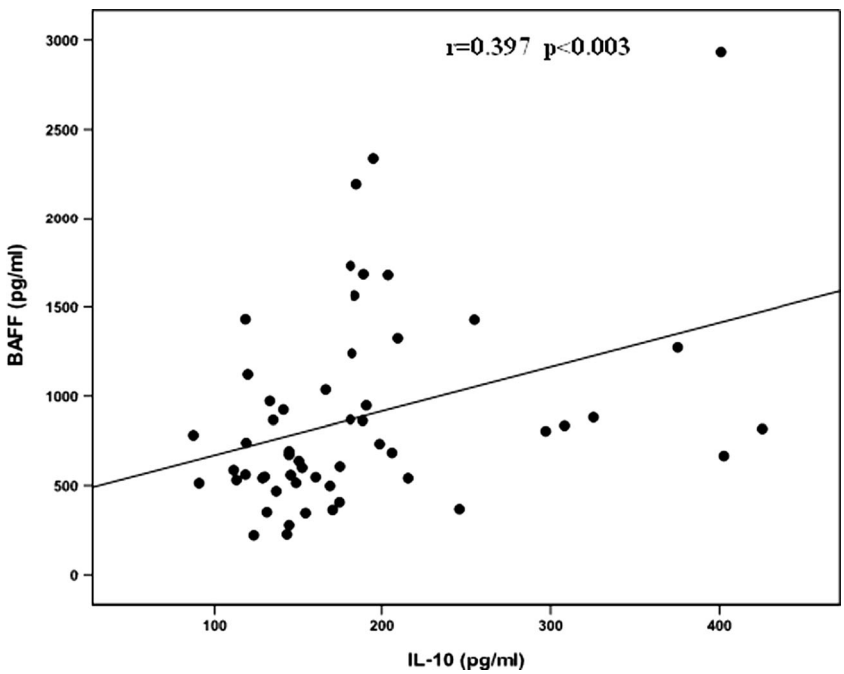

Fig 3 Positive correlation between serum levels of interleukin-10 (IL-10) with B-cell activating factor (BAFF), in multiple myeloma patients

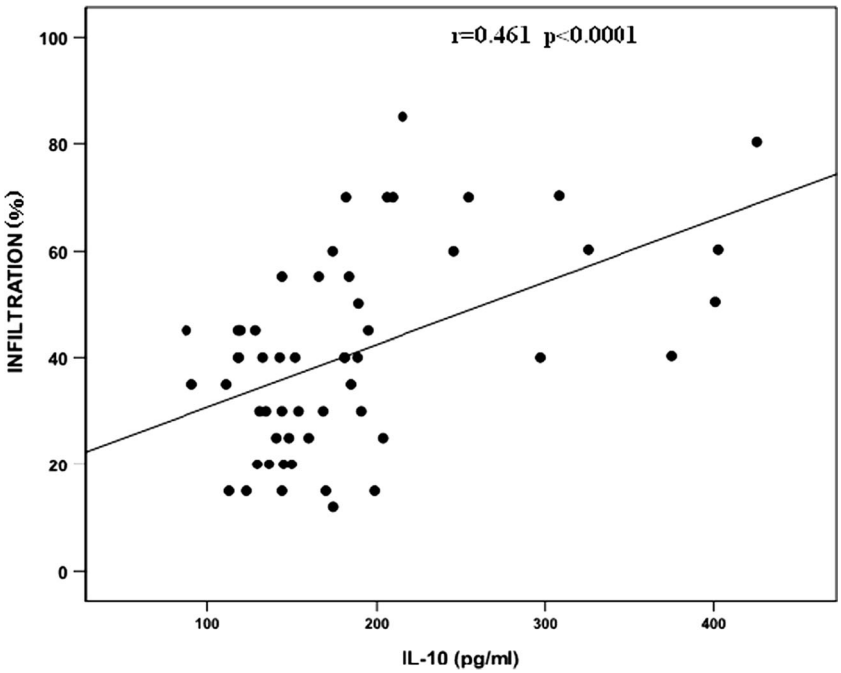

Fig 4 Positive correlation between serum levels of interleukin-10 (IL-10) with bone marrow infiltration by plasma cells, in multiple myeloma patients

In this study we also confirmed that both VEGF and Ang-2 serum levels were significantly higher in MM patients compared to controls and were increasing in parallel with the disease progression. These findings confirm the increased expression of VEGF and Ang-2 in myeloma cells and corroborate low levels of Ang-2 detected in patients with monoclonal gammopathy of undetermined significance and reactive plasmacytosis [34, 35].

In conclusion our results provide evidence that patients with active myeloma have raised serum levels of the angiogenic cytokines Ang-2 and VEGF, as well BAFF and IL-10. All these parameters strongly correlate to one another. The correlation of the angiogenic cytokines with IL-10, which in turn correlates with markers of disease activity, implies that they have an important role in MM pathogenesis. These findings support the hypothesis that VEGF, BAFF and Ang-2 might be used in future prognostic models and provide evidence that these cytokines could be a molecular target for MM drug development.

References

1. Kyle RA, Rajkumar SV (2004) Multiple myeloma. N Engl J Med 351:1860–1873
2. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420: 860–867
3. Benjamin D, Park CD, Sharma V (1994) Human B cell interleukin 10. Leuk Lymphoma 12:205–210
4. Lauta VM (2003) A review of the cytokine network in multiple myeloma: diagnostic, prognostic, and therapeutic implications. Cancer 97:2440–2452
5. Nacinović-Duletić A, Stifter S, Dvornik S, Skunca Z, Jonjic N (2008) Correlation of serum IL-6, IL-8 and IL-10 levels with clinicopathological features and prognosis in patients with diffuse large B-cell lymphoma. Int J Lab Hematol 30:230–239
6. Gupta M, Han JJ, Stenson M, Maurer M, Wellik L, Hu G, Ziesmer S, Dogan A (2012) Witzig TE (2012) Elevated serum IL-10 levels in diffuse large B-cell lymphoma: a mechanism of aberrant JAK2 activation. Blood 119:2844–2853
7. Fayad L, Keating MJ, Reuben JM, O'Brien S, Lee BN, Lerner S, Kurzrock R (2001) Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. Blood 97:256–263
8. Kovacs E (2010) Interleukin-6 leads to interleukin-10 production in several human multiple myeloma cell lines. Does interleukin-10 enhance the proliferation of these cells? Leuk Res 34:912–916
9. Alexandrakis MG, Roussou P, Pappa CA, Messaritakis I, Xekalou A, Goulidaki N, Boula A, Tsirakis G (2013) Relationship between circulating BAFF serum levels with proliferating markers in patients with multiple myeloma. Biomed Res Int 2013:389579
10. Vacca A, Ria R, Ribatti D, Semeraro F, Djonov V, Di Raimondo F, Dammacco F (2003) A paracrine loop in the vascular endothelial growth factor pathway triggers tumor angiogenesis and growth in multiple myeloma. Haematologica 88:176–185
11. Dankbar B, Padro T, Leo R, Feldmann B, Kropff M, Mesters RM, Serve H, Berdel WE, Kienast J (2000) Vascular endothelial growth factor and interleukin-6 in paracrine tumor-stromal cell interactions in multiple myeloma. Blood 95:2630–2636
12. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, Yancopoulos GD (1996) Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87:1161–1169
13. Scharpfenecker M, Fiedler U, Reiss Y, Augustin HG (2005) The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism. J Cell Sci 118:771–780
14. Holash J, Wiegand SJ, Yancopoulos GD (1999) New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. Oncogene 18:5356–5362
15. Papetti M, Herman IM (2002) Mechanisms of normal and tumor-derived angiogenesis. Am J Physiol Cell Physiol 282:C947–970
16. Giuliani N, Colla S, Lazzaretti M, Sala R, Roti G, Mancini C, Bonomini S, Lunghi P, Hojden M, Genestreti G, Svaldi M, Coser

P, Fattori PP, Sammarelli G, Gazzola GC, Bataille R, Almici C, Caramatti C, Mangoni L, Rizzoli V (2003) Proangiogenic properties of human myeloma cells: Production of angiopoietin-1 and its potential relationship to myeloma-induced angiogenesis. Blood 102:638–645

17. Fragioudaki M, Boula A, Tsirakis G, Psarakis F, Spanoudakis M, Papadakis IS, Pappa CA, Alexandrakis MG (2012) B cell-activating factor: Its clinical significance in multiple myeloma patients. Ann Hematol 91:1413–1418
18. Ria R, Reale A, De Luisi A, Ferrucci A, Moschetta M, Vacca A (2011) Bone marrow angiogenesis and progression in multiple myeloma. Am J Blood Res 1:76–89
19. Hao NB, Lü MH, Fan YH, Cao YL, Zhang ZR, Yang SM (2012) Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol 2012:948098
20. Sica A, Saccani A, Mantovani A (2002) Tumor-associated macrophages: a molecular perspective. Int Immunopharmacol 2:1045–1054
21. Mantovani A, Sica A, Allavena P, Garlanda C, Locati M (2009) Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. Hum Immunol 70:325–330
22. Sindrilaru A, Peters T, Wieschalka S, Baican C, Baican A, Peter H, Hainzl A, Schatz S, Qi Y, Schlecht A, Weiss JM, Wlaschek M, Sunderkötter C, Scharffetter-Kochanek K (2011) An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. J Clin Invest 121:985–997
23. Dace DS, Khan AA, Kelly J, Apte RS (2008) Interleukin-10 promotes pathological angiogenesis by regulating macrophage response to hypoxia during development. PLoS One 3:e3381
24. Lewis C, Murdoch C (2005) Macrophage responses to hypoxia: Implications for tumor progression and anti-cancer therapies. Am J Pathol 167:627–635
25. Solinas G, Germano G, Mantovani A, Allavena P (2009) Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J Leukoc Biol 86:1065–1073
26. Siveen KS, Kuttan G (2009) Role of macrophages in tumour progression. Immunol Let 123:97–102
27. Tanaka Y, Kobayashi H, Suzuki M, Kanayama N, Suzuki M, Terao T (2002) Thymidine phosphorylase expression in tumor-infiltrating macrophages may be correlated with poor prognosis in uterine endometrial cancer. Hum Pathol 33:1105–1113
28. Matsuda M, Salazar F, Petersson M, Masucci G, Hansson J, Pisa P, Zhang QJ, Masucci MG, Kiessling R (1994) Interleukin 10 pretreatment protects target cells from tumor- and allospecific cytotoxic T cells and downregulates HLA class I expression. J Exp Med 180: 2371–2376
29. Miotto D, Lo Cascio N, Stendardo M, Querzoli P, Pedriali M, De Rosa E, Fabbri LM, Mapp CE, Boschetto P (2010) CD8+ T cells expressing IL-10 are associated with a favourable prognosis in lung cancer. Lung Cancer 69:355–360
30. Lopez MV, Adris SK, Bravo AI, Chernajovsky Y, Podhajcer OL (2005) IL-12 and IL-10 expression synergize to induce the immune-mediated eradication of established colon and mammary tumors and lung metastasis. J Immunol 175:5885–5894
31. Mocellin S, Marincola FM, Young HA (2005) Interleukin-10 and the immune response against cancer: a counterpoint. J Leukoc Biol 78: 1043–1051
32. Berardi S, Ria R, Reale A, De Luisi A, Catacchio I, Moschetta M, Vacca A (2013) Multiple myeloma macrophages: pivotal players in the tumor microenvironment. J Oncol 183602
33. Vacca A, Ribatti D, Presta M, Minischetti M, Iurlaro M, Ria R, Albini A, Bussolino F, Dammacco F (1999) Bone marrow neovascularization, plasma cell angiogenic potential, and matrix metalloproteinase-2 secretion parallel progression of human multiple myeloma. Blood 93:3064–3073
34. Pappa CA, Tsirakis G, Samiotakis P, Tsigaridaki M, Alegakis A, Goulidaki N, Alexandrakis MG (2013) Serum levels of angiopoietin-2 are associated with the growth of multiple myeloma. Cancer Invest 31:385–389
35. Uneda S, Matsuno F, Sonoki T, Tniguchi I, Kawano F, Hata H (2003) Expressions of vascular endothelial growth factor and angiopoietin-2 in myeloma cells. Haematologica 88:113–115