PC Cell-derived Growth Factor (Granulin Precursor) Expression and Action in Human Multiple Myeloma

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ABSTRACT

Purpose: Multiple myeloma (MM) is a clonal B-cell neoplasm. PC cell-derived growth factor (PCDGF) is a Mr 88,000 glycoprotein growth factor. Our objective was to investigate the expression, function, and signaling pathways of PCDGF in human MM.

Experimental Design: PCDGF expression, function, and signaling pathways in human MM were studied using two human MM cell lines: ARP-1 and RPMI 8226. In addition, PCDGF expression in MM patients was examined using 13 human bone marrow biopsy samples.

Results: PCDGF mRNA and protein expression was detected in human MM cell lines such as ARP-1 and RPMI 8226. PCDGF added exogenously stimulated cell growth and sustained cell survival of both ARP-1 and RPMI 8226 cells in a dose- and time-dependent fashion. Conversely, treatment with neutralizing anti-PCDGF antibody inhibited the growth of RPMI 8226 cells suggesting that PCDGF acts as an autocrine growth factor for MM cells. Studies of signal transduction pathways showed PCDGF stimulated mitogen-activated protein kinase and phosphatidylinositol 3'-kinase pathways but not the Janus-activated kinase-signal transducer and activator of transcription pathway. Immunohistochemical analysis of bone marrow smears obtained from MM patients indicated that PCDGF expression was associated with myeloma cells from MM patients and correlated with the presence of MM disease.

Conclusion: These data suggest that PCDGF is an autocrine growth factor in the cell growth and survival of human MM cells, and may be a potential candidate as a biomarker of MM cells.

INTRODUCTION

MM is a clonal plasma cell neoplasm that results in the accumulation of malignant plasma cells (1). MM currently represents 10–15% of all hematological neoplasms in the United States. There are ~14,400 new cases of MM diagnosed per year and 11,200 deaths in the United States (2). The overall incidence rate in the United States is 4 of 100,000 persons per year (3). There has been little improvement in the treatment of human MM over the past 25 years, and there is no cure for the disease (4). The choice of therapy for human MM is limited. The standard treatment for human MM is chemotherapy with melphalan and prednisone alone or combinations of alkylating agents, glucocorticoids, and anthracyclines (5). In addition, nearly all of the patients with MM who showed an initial response to glucocorticoid therapy relapse and progress to more aggressive forms of the disease that become insensitive to the killing effect of glucocorticoid. The median survival of MM patients treated with conventional chemotherapy is ~3 years. To overcome the resistance of MM cells to conventional chemotherapy, autologous/allogeneic/syngeneic hematopoietic stem cell transplantation with high-dose therapy was introduced in the treatment for MM patients. This method appears to be superior to conventional chemotherapy in terms of event-free survival and overall survival. But the condition of MM patients eventually relapses because transplantation does not completely eliminate MM cells. Thalidomide was reported recently as an effective therapy in MM patients (6, 7). The exact mechanism of thalidomide is unknown. Several models have been proposed including angiogenesis inhibition by down-regulation of vascular endothelial growth factor, immune modulation by increasing natural killer cell activity, IL-2 and IFN-γ, and increasing apoptosis (8). However, despite these recent advances, future progress in treatments of MM is dependent on the identification of novel biological molecular targets of importance for the cell growth and survival of MM. Once identified, such targets can provide potential leads for the understanding of the causes of the disease, and provide novel biomarkers for diagnosis and therapy (9). Studies of the biology and pathogenesis of human MM have implicated the importance of autocrine and paracrine growth regulation in MM. The best characterized growth factor is IL-6, which has been identified as a major growth factor for MM cells. IL-6 produced by bone marrow stromal cells, osteoblasts, and osteoclasts in the microenvironment of the bone marrow may operate a paracrine mechanism, whereas MM cells them-
selves may produce autocrine IL-6 (1, 10). IL-6 activates JAK-STAT and MAP kinase signaling pathways (1, 11). In addition, IL-6 was demonstrated recently to stimulate PI3k signaling pathways in human MM (12, 13).

PCDGF, also known as progranulin, is a Mᵦ 88,000 glycoprotein composed of a Mᵦ 68,000 protein core and a Mᵦ 20,000 carbohydrate moiety, originally isolated as an autocrine growth factor from the culture medium of the highly tumorigenic mouse teratoma-derived cell line PC (14, 15). Amino acid and cDNA sequencing indicated that PCDGF was identical to the precursor of epithelins/granulins (16, 17). In addition to being overexpressed in mouse teratoma-derived cells (14), several laboratories including ours showed that PCDGF was specifically overexpressed in many tumor types including glioblastoma (18), renal clear cell carcinoma (19), and human breast cancer cells (20). PCDGF was demonstrated as a growth stimulator for a variety of cell lines including fibroblasts, teratoma PC cells, and mammary epithelial cells (14, 20–22). Blocking of PCDGF action by neutralizing anti-PCDGF antibody showed that PCDGF acted as an autocrine growth factor for mouse teratoma-derived cells and human breast cancer cells (15, 23). Most importantly, inhibition of PCDGF expression by antisense transfection led to complete inhibition of tumorigenesis for both mouse teratoma-derived cells and human breast cancer cells injected in nude mice (15, 23). In the present paper, we investigated for the first time the expression and function of PCDGF in human MM.

MATERIALS AND METHODS

Cell Lines and Reagents. Human B-cell lines Daudi and Raji, human MM cell line RPMI 8226, human T-cell line Jurkat, and human promyelocytic leukemia cell line HL-60 were obtained from the American Type Culture Collection (Manassas, VA). Human MM cell line ARP-1 was established at the Arkansas Cancer Research Center from bone marrow aspirates of patients with MM (24) and was provided by Dr. Robert G. Fenton (University of Maryland, School of Medicine, Baltimore, MD). Human T-cell line KOPT-K1 was a gift from Dr. Taijiro Mori (Keio University, Tokyo, Japan). RPMI 1640 and TRIzol were obtained from Invitrogen Life Technologies, Inc. (Carlsbad, CA). Alexa 456-conjugated goat antirabbit IgG F(ab’2) and Alexa 488-conjugated goat antimouse IgG were obtained from Molecular Probes (Eugene, OR). Human recombinant IL-6 was obtained from Upstate Biotechnology Inc. (Lake Placid, NY). PD98059, antiphsoph-MAP kinase antibody, antiphosph-Akt antibody, anti-Akt antibody, and antiphosph-tyr-STAT3 were obtained from New England Biolabs (Beverly, MA). Anti-STAT3 was obtained from BD Biosciences (San Jose, CA). Anti-MAP kinase antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). LY294002 and wortmannin were obtained from Biomol (Plymouth Meeting, PA). Supersignal Western chemiluminescent substrate was obtained from Pierce (Rockford, IL). Immobilon-P transfer membranes were obtained from Millipore (Bedford, MA). Monoclonal antibodies to antihuman κ or λ light chains were obtained from Dako (Carpinteria, CA). Protein A-Sepharose was obtained from Amersham Pharmacia Biotech (Piscataway, NJ). Human PCDGF and antihuman PCDGF antibody were prepared in our laboratory (25). All of the other reagents were obtained from Sigma (St. Louis, MO).

Neutralization of PCDGF Produced by MM Cells. RPMI 8226 cells were cultured in 10% FBS supplemented RPMI 1640. Before the assay, cells were washed with RPMI 1640 and cultured in RPMI 1640 at 2.5 × 10⁴ live cells/ml. After 24 h, PCDGF or IL-6 was added to medium at the indicated concentrations. Determination of cell density and viability was carried out after 24 and 48 h of treatment by counting live and total cell densities with a hemocytometer using trypan blue exclusion assay. The percentage of survival was calculated by live cell density/total cell density.

Determination of MAP Kinase, Akt, and STAT3 Phosphorylation. ARP-1 and RPMI 8226 cells were cultured in 10% FBS-supplemented RPMI 1640. Before assays, cells were washed with RPMI 1640 and cultured in RPMI 1640 at 2.5 × 10⁴ live cells/ml. After overnight starvation, cells were treated with or without 30 μM PD98059 for 60 min to determine MAP kinase phosphorylation, with or without 50 μM LY294002 for 10 min to determine Akt phosphorylation. Then PCDGF was added at different concentrations as indicated in the figures. For determination of STAT3 phosphorylation, 10 ng/ml IL-6 was used as a positive control. After 10–15 min incubation, cells were lysed in Laemmli sample buffer. Lysate from 3 × 10⁴ live cells was used for each sample. Proteins were separated on a 12.5% SDS-PAGE for MAP kinase and Akt, and on a 7.5% SDS-PAGE for STAT3. Phosphorylated and total form of MAP kinase, Akt, or STAT3 proteins were detected by Western blot analysis using appropriate antibodies.

Immunohistochemistry Studies. Bone marrow smears obtained from MM patients at the University of Maryland Greenebaum Cancer Center were fixed for 15 min on ice with 2% paraformaldehyde in PBS. After several washes with PBS, the cells were permeabilized with PBS containing 0.2% Triton X-100 for 15 min at room temperature. The slides were stained with rabbit antihuman PCDGF antibody at room temperature for 1 h. After washing with PBS, samples were incubated with Alexa 488-conjugated goat antirabbit IgG F(ab’)₂ at room temperature for 1 h. The slides were also stained with antihuman κ or λ light chains monoclonal antibodies at room temperature for 1 h. After washing with PBS, samples were incubated with 1 μg/ml Alexa 456-conjugated goat antimouse IgG F(ab’)₂ at room temperature for 1 h. Finally samples were stained by 0.5
μg/ml DAPI at room temperature for 15 min. Stained bone marrow samples were observed with Olympus BX40 fluorescence microscope equipped with 100-W mercury lamp and appropriate filters.

Determination of PCDGF Protein Expression. Daudi, Raji, ARP-1, RPMI 8226, Jurkat, KOPT-K1, and HL-60 cells were cultured at a density of 1 × 10^6 cells/ml in RPMI 1640 supplemented with 10% FBS. When the cells reached a density of 1 × 10^6 cells/ml, the culture medium containing 1.5 × 10^7 live cells were collected to determine the level of PCDGF produced in the medium followed by immunoprecipitation and Western blot analysis. All of the samples analyzed were normalized to the same cell number. Conditions for immunoprecipitation and Western blot analysis have been described previously (20) except that we used 50 μg/ml anti-PCDGF horseradish peroxidase conjugate for the Western blot analysis.

Statistics. Experiments were carried out in triplicates and repeated three times. The results were expressed as mean ± SD.

RESULTS
Expression of PCDGF in Two Human MM Cell Lines: ARP-1 and RPMI 8226. To check PCDGF expression in human MM cells, we examined the expression of PCDGF mRNA and protein in ARP-1 and RPMI 8226 cells by Northern and Western blot analysis, respectively. As shown in Fig. 1, PCDGF mRNA and protein were expressed in both cell lines. PCDGF mRNA expression level was equivalent in these cells. However, the level of PCDGF protein produced in the culture medium was higher in RPMI 8226 than in ARP-1 cells.

Effect of PCDGF on Cell Growth and Survival of Human MM Cell Lines. The effect of exogenously added PCDGF on the growth and survival of MM cells was examined using ARP-1 and RPMI 8226 cells. IL-6, a known major growth factor of MM cells (1), was used as a positive control in these experiments. As shown in Fig. 2A, after 24 h of treatment, 50, 100, and 200 ng/ml PCDGF, and 10 ng/ml IL-6 stimulated ARP-1 cell growth by 1.3-, 1.5-, 1.5-, and 1.6-fold, respectively. After 48 h treatment, 50, 100, and 200 ng/ml PCDGF, and 10 ng/ml IL-6 stimulated RPMI 8226 cell growth by 1.7-, 2.5-, 2.6-, and 2.8-fold, respectively (Fig. 2C). These data show that PCDGF stimulated the growth of ARP-1 cell and RPMI 8226 cells in a dose- and time-dependent fashion, and reached a fold stimulation similar to the one observed with IL-6. In addition to stimulating the growth of MM cells, exogenously added PCDGF sustained cell survival of ARP-1 and RPMI 8226 cells in a dose- and time-dependent fashion (Fig. 2, B and D). When added at suboptimal concentrations, PCDGF and IL-6 showed additive effect on ARP-1 and RPMI 8226 cell growth and survival (data not shown).
Effect of Anti-PCDGF Neutralizing Antibody on the Growth of RPMI 8226 Cells. Because PCDGF is produced by MM cells, experiments were carried out to test whether PCDGF acts as an autocrine factor for these cells. For this purpose, we examined the effect of anti-PCDGF neutralizing antibody on the growth of RPMI 8226 cells. We had shown previously the neutralizing effect of anti-PCDGF antibody on the cell growth of breast cancer cells that overexpress PCDGF (23). As shown in Fig. 3, treatment of RPMI 8226 cells with 200 μg/ml affinity purified anti-PCDGF antibody resulted in a 50% inhibition of RPMI 8226 cell growth. The addition of 200 ng/ml PCDGF in the culture medium reversed the inhibitory effect of anti-PCDGF antibody. Combined with the previous results, this result shows that PCDGF acts as an autocrine growth factor for MM cells.

Signaling Pathways Stimulated by PCDGF in Human MM Cells. PCDGF has been shown to activate MAP kinase pathway in human breast cancer MCF-7 cells (25) and activate both MAP kinase and PI3k pathways in mouse embryo fibroblasts R-cells that lack IGF-I receptors and in adrenal carcinomas SW-13 cells (26, 27). MAP kinase activity is stimulated in response to many different growth factors, and MAP kinase signaling pathway plays a key role in cell proliferation process (28, 29). PI3k signaling pathway is mainly associated with survival and cell growth regulation (30, 31). We examined whether PCDGF added exogenously stimulated MAP kinase pathway in ARP-1 and RPMI 8226 cells. As shown in Fig. 4, A and B, the effect of PCDGF on ARP-1 cell growth and survival was blocked by the MAP/extracellular signal-regulated kinase (ERK) kinase inhibitor PD98059 at 30 μM. Similar result was observed in RPMI 8226 cells (Fig. 4, C and D). As shown in Fig. 5A, PCDGF showed a dose-dependent effect on phosphorylation of Erk1 and Erk2 in ARP-1 cells. This dose-dependent phosphorylation correlated with PCDGF effect on cell growth (Fig. 2). The phosphorylation of Erk1 and Erk2 by optimal concentration of PCDGF (200 ng/ml) in ARP-1 cells was inhibited by 30 μM PD98059 (Fig. 5B). Similar result was observed in RPMI 8226 cells (Fig. 5C). These results suggested that PCDGF activated MAP kinase pathway in MM cells and that MAP kinase responded for stimulation of cell growth and sustentation of cell survival by PCDGF. Concerning the PI3k signaling pathway,
we checked the Akt phosphorylation that occurs downstream of PI3k. PI3k generates phosphatidylinositol-3,4,5-trisphosphate (3), a lipid second messenger essential for the translocation of Akt to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase-1 and possibly other kinases (32). As shown in Fig. 6A, PCDGF stimulated the phosphorylation of Akt in ARP-1 cells, and this phosphorylation was inhibited by PI3k inhibitor LY294002 at 50 μM. These results suggested that PCDGF activated PI3k-Akt pathway in ARP-1 cells. We also checked the Akt phosphorylation that occurs downstream of PI3k; P-Akt, phosphorylated Akt; P-STAT3, phosphorylated STAT3.

![Fig. 6](image)

**Fig. 6** PCDGF induced phosphorylation of Akt but not of STAT-3 in ARP-1 cells. Samples were treated as described “Materials and Methods.” Cell lysates from 3 x 10⁵ ARP-1 cells were loaded per lane and blotted with anti-phosph-Akt or anti-phosph-STAT3. The same membrane was stripped and rebotted with anti-Akt or anti-STAT3 to indicate equal loading. A, PCDGF effect on phosphorylation of Akt in ARP-1 cells. B, PCDGF effect on phosphorylation of STAT3 in ARP-1 cells. C, control; P, 200 ng/ml PCDGF; IL-6, 10 ng/ml IL-6; LY, 50 μM LY294002; P-Akt, phosphorylated Akt; P-STAT3, phosphorylated STAT3.

Table 1

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![Fig. 7](image)

**Fig. 7** Determination of PCDGF expression in bone marrow smears of patients with MM. A, anti-human κ chain antibody (A), anti-human γ/δ chain antibody (B), and anti-human PCDGF antibody (C). The left bottom cell represents a myeloma cell that shows positive DAPI, human κ/λ chain, and human PCDGF staining. The right top cell represents a macrophage cell that shows negative human κ/λ chain and human PCDGF but positive DAPI staining.  

was detected in the MM cells in bone marrow samples (5b in Table 1). A typical triple staining by DAPI, κ/λ chain, and PCDGF is shown in Fig. 7. As shown in Fig. 7, the left bottom cell is a myeloma cell that shows positive DAPI, human κ/λ chain, and human PCDGF staining. The right top cell is a macrophage cell that shows negative human κ/λ chain and human PCDGF but positive DAPI staining. These data clearly indicated that PCDGF expression is associated with myeloma cells from all of the MM patients examined and correlated well with the presence of the disease.

**PCDGF Expression in Human Leukemic Cell Lines.** We examined PCDGF expression in several types of human leukemic cell lines of B- or T-cell origin to determine whether PCDGF expression is limited to MM. Samples examined were standardized to the same cell number as described in “Materials and Methods.” As shown in Fig. 8, PCDGF protein expression was detected in human B-cell lines (Raji and Daudi) as well as in human MM cell lines (ARP-1 and RPMI 8226). In contrast,
PCDGF protein expression was not detected in human T-cell lines (Jurkat and KOPT-K1) and promyelocytic leukemia cell line (HL-60). These results suggested that PCDGF is preferentially expressed by hematological malignancies of the B-cell lineage.

DISCUSSION

The identification of biological molecular targets responsible for the cell growth and survival of MM is important. Once identified, such targets can provide potential leads for understanding the disease, and for developing new prognosis factors and specific therapy (9). Here we provide evidence that PCDGF is expressed in MM and acts as an autocrine growth factor for these cells. There have been several growth factors reported as stimulators of MM cell growth or survival. IL-6, which is produced by cells constituting the bone marrow microenvironment and MM cells, is a major growth factor for MM. Other members of the IL-6 type cytokine family including IL-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotoxin-I also have been shown to act as growth factors for human MM (34).Granulocyte colony-stimulating factor, IL-1, and tumor necrosis factor also stimulate the growth of MM cells (34). In contrast, IFNs, IL-2, and IL-4 often exert growth inhibitory effects on myeloma cells (34). IGF-I has emerged recently as a possible autocrine growth factor for human MM (35). Expression of IGF-I and its receptor has been demonstrated in several human MM cell lines (35, 36). IGF-I stimulated the proliferation of several human MM cell lines and significantly augmented IL-6 stimulated proliferation (37). In contrast, IGF-I did not directly stimulate normal B-cell proliferation, nor did it augment the activity of the known B-cell stimulators of MM cell growth or survival. IL-6, which is produced by cells constituting the bone marrow microenvironment and MM cells, is a major growth factor for MM. Other members of the IL-6 type cytokine family including IL-11, leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and cardiotoxin-I also have been shown to act as growth factors for human MM (34). Granulocyte colony-stimulating factor, IL-1, and tumor necrosis factor also stimulate the growth of MM cells (34). In contrast, IFNs, IL-2, and IL-4 often exert growth inhibitory effects on myeloma cells (34). IGF-I has emerged recently as a possible autocrine growth factor for human MM (35). Expression of IGF-I and its receptor has been demonstrated in several human MM cell lines (35, 36). IGF-I stimulated the proliferation of several human MM cell lines and significantly augmented IL-6 stimulated proliferation (37). In contrast, IGF-I did not directly stimulate normal B-cell proliferation, nor did it augment the activity of the known B-cell growth factor, IL-2, in the presence or absence of Staphylococcus aureus (37). In human MM cells, IGF-I activated MAP kinase and PI3k signaling pathways, leading to both proliferative and antiapoptotic effects (38). We show here for the first time that PCDGF is expressed in human MM and acts as an autocrine growth factor for human MM. PCDGF expression was also detected in mouse MM cell lines but not in mouse normal B cells.4

When we examined the expression of PCDGF in ARP-1 and RPMI 8226, we found the PCDGF mRNA expression level was equivalent in these cells; however, the PCDGF protein level secreted into the culture medium is higher in RPMI 8226 than in ARP-1 cell. This could be because of different regulations of PCDGF translation/secretion or different stabilities of PCDGF mRNA/protein in these two cell lines.

When we examined PCDGF expression profile in hematological cell lines, we found that PCDGF protein was detected in hematological malignancies of the B-cell lineage (B cell lines and MM cell lines) but not in T-cell lineage (Jurkat and KOPT-K1) and promyelocytic leukemia (HL-60). It has been reported previously that HL-60 cells express granulin precursor mRNA (39). In our experiments, we checked the PCDGF protein secreted to culture medium. The data show that secretion of PCDGF protein by HL-60 cells could not be detected. The reason for the lack of correlation between PCDGF mRNA expression and protein secretion could because of post-transcriptional regulation of PCDGF protein secretion that would make PCDGF secreted by HL-60 cells below the detectable level. Nonetheless, the studies presented here compare the level of PCDGF protein secreted by the various hematological malignancies point out that high PCDGF expression is preferentially found in the B-cell leukemia lineage. It should be pointed out that the only form detected in the culture medium was the Mr 88,000 PCDGF corresponding to the granulin precursor (14). We could not detect in the culture medium smaller processed granulin forms, particularly the Mr 6,000 granulins (also called epithelins 1 and 2) that have first been purified from granulocyte or kidney extracts (40, 41). PCDGF was identified as 1 of 30 most up-regulated transcripts between AML cells with isolated trisomy 8 (AML +8) and CD34+ cells that were purified from normal bone marrow as a representative heterogeneous population of stem and progenitor cells (42). Interestingly, PCDGF was one of top 50 genes effective in discriminating between acute lymphoblast leukemia and AML when measured by median vote relevance (43). It was reported that PCDGF mRNA is expressed in normal immune cells (39). However, the PCDGF function in immune-derived cells remains unknown. In this present paper, we reported that PCDGF is expressed by human MM cells and is required to stimulate their proliferation. The acquisition of an autocrine production of PCDGF maybe one of the necessary steps for B-cell transformation or for maintenance of the tumorigenic phenotype. In support of this later possibility, PCDGF expression has been shown to be essential for human breast carcinoma tumorigenesis (23). It will be interesting to see if this is the case for MM cells. Moreover, it will be interesting to investigate how PCDGF expression is regulated in different hematological cell lines and whether PCDGF has effects on B-cell lineage besides the effects on cell growth and survival.

We examined the signaling pathways stimulated by PCDGF in human MM. Similar to what was found with PCDGF in human breast cancer cells (25), mouse embryonic fibroblast R-cells (26), and SW-13 cells (27), MAP kinase pathway plays an important role in PCDGF function in human MM. In support of this observation, PCDGF also stimulated MAP kinase phosphorylation, and the addition of the MAP/extracellular signal-regulated kinase inhibitor PD98059 inhibited the activity of PCDGF to stimulate MM cell growth and cell viability. We found that PCDGF stimulated PI3k pathway, as shown by stimulation of Akt phosphorylation in human MM. However, we could not demonstrate whether PCDGF stimulation of PI3k

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pathway is involved in cell growth and survival, because PI3k inhibitor (LY294002 or Wortmannin) has a high cytotoxic effect on ARP-1 and RPMI 8226 cells even in the absence of PCDGF. Other approaches such as using dominant-negative mutants blocking PI3k pathway were not feasible, because MM are very difficult to transfect with a reasonable efficiency. JAK-STAT signaling pathway, which is stimulated by IL-6, did not appear to be activated by PCDGF in human MM. Similar results were found with IGF-I in human MM (44).

Strong emphasis has been placed on finding new biomarkers for human MM. Plasma cell labeling index, β2-microglobulin, soluble IL-6 receptor, C-reactive protein, creatinine, and plasma cell percentage by immunofluorescence analysis of liquid marrow have been accepted as biomarkers (5). A new report recently showed serum syndecan-1 as a new independent biomarker in MM (45). Until now, there has not been any growth factor shown to act as a biomarker for MM. Serum IL-6 level has been shown not to have a prognostic role in MM (46). Our immunohistochemistry study indicates that PCDGF is expressed in bone marrow smears by MM patients, whereas it is absent in patients in remission. These data suggest that PCDGF may be a potentially excellent candidate as a biomarker for this disease. Detailed studies are necessary to additionally examine the possible correlation between PCDGF level and the progression of MM disease.

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REFERENCES

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