Functional Role of Cation-Independent Mannose 6-Phosphate/Insulin-Like Growth Factor II Receptor in Cell Adhesion and Proliferation of a Human Myeloma Cell Line OPM-2

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The molecular mechanism underlying the interaction between myeloma cells and stromal cells was investigated by using a human myeloma cell line (OPM-2) and human umbilical vein endothelial cells (HUVECs). Adhesion of OPM-2 cells to HUVECs was found to be significantly augmented with treatment of OPM-2 cells with an α-glycosidase inhibitor, castanospermine (CSP). The treatment of OPM-2 cells with CSP resulted in alteration of oligosaccharide structures of cell surface glycoproteins particularly at molecular weight of 220 kD (GP220). To determine if GP220 was involved in the adhesion of OPM-2 cells to HUVECs, cell surface glycoproteins of HUVECs were labeled by biotin and were incubated with the PVDF membrane to which cell surface glycoproteins of OPM-2 cells were biotinylated. The biotinylated glycoproteins at the plasma membrane of HUVECs specifically bound to GP220 of OPM-2 cells. Purification and partial amino acid sequencing of GP220 revealed that GP220 had a structure homologous to cation-independent mannose 6-phosphate/insulin-like growth factor-II (CIM6P/IGF-II) receptor. Furthermore, an antibody against CIM6P/IGF-II receptor was reactive with GP220, indicating that GP220 was a CIM6P/IGF-II receptor. The adhesion of OPM-2 cells to HUVECs was inhibited by mannose 6-phosphate. Moreover, M6P was found to suppress the adhesion of human myeloma cell lines, OPM-2 and RPMI 8226, to bone marrow stromal cells that was established from the patients with multiple myeloma. In addition, proliferation of OPM-2 was stimulated in response to IGF-II. These results suggest that CIM6P/IGF-II receptor may be functional in terms of supporting cell adhesion and proliferation of myeloma cells.

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MULTIPLE MYELOMA is a human B-cell neoplasia characterized by clonal expansion of malignant plasma cells that secrete monoclonal immunoglobulins and by multiple osteolytic lesions. A number of previous studies have suggested that multiple cytokines such as interleukin-6 (IL-6), IL-1, and tumor-necrotizing factor-β play a role in the abnormal growth of myeloma cells and also in the osteoclastic bone destruction. However, the proliferation of the freshly prepared myeloma cells from the myeloma patients is not consistently supported by IL-6 alone or by IL-6 in the combination with other cytokines. Because myeloma cells proliferate predominantly in bone marrow, it is suggested that the growth and localization of myeloma cells in bone marrow may be supported by their direct interaction with bone marrow stromal cells or extracellular matrix molecules. In fact, myeloma cells have been shown to express very late antigen-4 (VLA-4) and CD44 antigen, which are adhesion molecules responsible for the interaction between myeloma cells and stromal cells have not yet been fully elucidated.

Cell adhesion molecules are glycoproteins in which carbohydrates are either N- or O-linked depending on to which residues they are linked. N-linked chains are more common than O-linked ones. N-linked chains are added to the growing peptide chain in the endoplasmic reticulum, and N-linked carbohydrate chains are modified in the Golgi apparatus to acquire either high-mannose-, hybrid-, or complex-type motifs. Despite much information available concerning the structure and synthesis of N-linked oligosaccharides, the biological significance of the oligosaccharides in the function of cell adhesion molecules is just coming to be realized. The carbohydrate structures of glycoproteins can be modified by specific inhibitors of carbohydrate processing, ie, castanospermine (CSP), deoxynojirimycin, swainsonine, which produce different kinds of blocked carbohydrate chains. CSP inhibits α-glycosidase, which is involved in the initial step of N-linked oligosaccharide processing of glycoproteins, thereby increasing the high-mannose type on cell surface glycoproteins of the treated cell. In a previous study, we showed that CSP inhibited pokeweed mitogen-induced B-cell maturation through the reduced adhesion of B cells to T cells. Because such inhibitors have been shown to affect the expression or the function of adhesion molecules through the alteration of oligosaccharides, they may be useful not only for understanding the role of the oligosaccharide structures in cell adhesion but also for identifying molecules involved in cell-cell interaction. In an effort to clarify the molecular mechanisms underlying the interaction between myeloma cells and stromal cells, cells of a human myeloma cell line (OPM-2) were treated with or without CSP, and the adhesion of the OPM-2 cells to human umbilical vein endothelial cells (HUVECs) was measured. The treatment of OPM-2 cells with CSP led to a significant increase in adhesion of the cells to HUVECs. The CSP treatment of OPM-2 cells also resulted in striking alteration in oligosaccharide structures of a glycoprotein at molecular weight of 220 kD (GP220). By using a new ligand-blotting technique, biotin-labeled proteins at the plasma membrane of HUVECs were found to specifically bind to GP220. The amino acid sequence of purified GP220 revealed that GP220
was cation-independent mannose 6-phosphate/insulin-like growth factor-II (CIM6P/IGF-II) receptor. Furthermore, the adhesion of OPM-2 cells to HUVECs was inhibited by mannose 6-phosphate, and the growth of OPM-2 cells was stimulated by IGF-II via its interaction of IGF-II receptor. Thus, our data provide the first evidence for role of CIM6P/IGF-II receptor in cell adhesion and growth regulation of myeloma cells.

**MATERIALS AND METHODS**

**Reagents.** Anti-VA44α (CD49d) and anti-VA5β (CD49e) monoclonal antibodies (MoAbs) were kind gifts of Dr Kensuke Miyake (Saga Medical School, Saga, Japan). Anti-β2 integrin (CD18) MoAB, anti-LFA1 (CD11a) MoAb, anti-CD54, and anti-LAM-I yake (Saga Medical School, Saga, Japan). Anti-P2 integrin (CD18) was kindly provided by Dr Stuart Komfeld (Washington University, St Louis, MO). Purified murine myeloma protein of IgGl (MOPC21) was purchased from Cappel (Durham, NC). Chondroitinase ABC (Sigma Chemical Co, St Louis, MO) was used as substrate for the assay of Crumton et al.17 Briefly, the cell lysates of biotin-labeled HUVECs were eluted with the lysate buffer containing 0.2 mol/L galactosyl-1-propanesulfonate containing 0.15 U/mL aprotinin (Sigma) and 0.05% Tween 20 (TBST). After washing three times with TBST and was incubated with TBS containing horseradish peroxidase-conjugated streptavidin (Zymed, San Francisco, CA). The separated proteins were electrophoretically transferred to PVDF membrane. To avoid nonspecific binding, PVDF membrane was subsequently incubated with 1% gelatin in Tris-buffered saline containing 0.05% Tween 20 and 10 mM MgCl2.

**Ligand blotting.** The cell lysate of OPM-2 cells (5 x 106 cells) was subjected to SDS-PAGE. The separated proteins were electrochemiluminescence Western blot detection system (Amersham, Arlington Heights, IL).

**Oligosaccharide analysis.** The OPM-2 cells and CSP-treated OPM-2 cells were lysed in the lysis buffer (20 mM/L Tris-HCl, pH 8.0, 137 mM/L NaCl, 1% 3-(3-Cholamidopropyl) dimethylammonio)-1-propanesulfonate containing 1 mM/L phenyl methylsulfonyl fluoride (Sigma) and 0.15 U/mL aprotinin (Sigma) at 4°C for 20 minutes. Insoluble material was removed by 10,000g centrifugation at 4°C for 15 minutes. The protein contents were determined with a BCA protein assay reagent (Pierce, Rockford, IL) using bovine serum albumin as a standard. The cell lysates (10 μg protein) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure of Laemmli18 and transferred to polyvinylidene difluoride membrane (PVDF; Immunobilon; Millipore, Bedford, MA). For oligosaccharide analysis, the transferred protein was stained with avidin biotin methods using biotinylated Recinus communis agglutinin (RCA, 1 μg/mL; EY Laboratories, Inc, San Mateo, CA) or biotinylated concanavalin A (Con A, 1 μg/mL; Vector Laboratories, Inc, Burlingame, CA) as described.19

**Cell surface labeling.** OPM-2 cells and HUVECs were surface-labeled by sulfo-NHS-biotin (Pierce) as previously described.20 Bio- tin-labeled cells were lysed with the lysis buffer. The glycoproteins at the plasma membrane of HUVECs were obtained by the methods of Crumton et al.21 Briefly, the cell lysates of biotin-labeled HUVECs were applied to the column of Lens culinaris lectin coupled of agarose (EY Laboratories, Inc, San Mateo, CA). The bound glycoproteins were eluted by the procedures of the manufacturer’s instructions. The eluted fractions were concentrated by use of Centricron (Kurabo, Kurashiki, Japan). The buffer of this sample was exchanged for phosphate-buffered saline (PBS) containing 0.05% Tween 20 and 10 mM/L MgCl2.

**Preparation and culture of HUVECs.** HUVECs were obtained from the veins of umbilical cords by modification of the technique described by Jaffe et al.15 HUVECs were cultured in TC 199 medium (Osaka Biken, Osaka, Japan) supplemented with 10% fetal calf serum. HUVECs were cultured in 100-mm dishes for 2 days at 37°C in a humidified atmosphere containing 5% CO2.
Blue R-250 in 50% methanol:10% acetic acid and detained. The band of GP220 was cut and stored at -20°C. After removal of the detained solution, each lane was cut out and incubated with 100 mmol/L Tris-HCl (pH 9.0) containing 0.1% SDS for 1 hour at 37°C and then endoproteinase Lys-C (Promega Corp, Madison, WI) was added at a concentration greater than 2 μg/mL. Protease digestion was carried out overnight at 37°C. The digestion solution was aspirated and reserved (first extract). To extract peptides from gels, the gels were crushed by centrifuge methods described by Kobayashi et al. A fivefold volume of 100 mmol/L Tris-HCl (pH 9.0) containing 0.1% SDS was added to crushed gels, and an extraction was performed by shaking gently for about 1 hour at room temperature. After incubation, the crushed gel was removed using a Millipore Ultrafree C3GV (UFCH3GV00, 0.22 μm pore). The second extract was added to the first extract, and the total extract was subjected to tricine-SDS-PAGE according to the procedure of Ploug et al. Polypeptides were blotted onto a PVDF membrane, which was then stained with Coomassie Blue essentially as described by Matsuda. The polypeptide band was cut out and stored in an Eppendorf tube at -20°C. Sequencing was carried out and stored in an Applied Biosystems 477A Protein Sequencer (Foster City, CA).

**Immunoprecipitation and immunoblotting.** OPM-2 cells and CSP-treated OPM-2 cells were surface-labeled by sulfo-normal human serum-biotin as described above. The lysates of OPM-2 cells and CSP-treated OPM-2 cells were precleared with 5 μL of normal rabbit serum and protein A-Sepharose beads (Pharmacia, Uppsala, Sweden) for 2 hours at 4°C. The precleared lysates were then incubated with 5 μL of rabbit anti-CIM6P receptor antisera and protein A-Sepharose beads to collect the antigen-antibody complexes. The immunoprecipitates were washed five times with lysis buffer containing proteinase inhibitors as described above and subjected to SDS-PAGE. After transfer to PVDF membrane and blocking with TBST containing 0.1% gelatin, immunoprecipitated surface proteins were detected with horseradish peroxidase-streptavidin (Zymed) and the electrochemiluminescence Western blot detection system.

The GP220 fraction of OPM-2 cells (10 μg protein) was subjected to SDS-PAGE, and was electrophoretically transferred to PVDF membrane. After blocking of the membrane with 1% gelatin in TBST as described above, the membrane was incubated overnight with rabbit anti-CIM6P receptor antibody serum. Immunoblotting was performed with alkaline phosphatase-conjugated antirabbit IgG (Promega) as previously reported.

**Proliferation assay.** To quantitate the proliferation of OPM-2 cells, we used MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma] rapid colorimetric assay as previously described. OPM-2 cells were cultured at a final concentration of 1 x 10^3/mL (1 x 10^3 in 100 μL) in Cosmedium 003 in 96-well tissue culture plates for 2 days. Where indicated, IGF-II (2 to 200 ng/mL; Genzyme, Cambridge, MA) was added to these cultures. An anti-IGF-I receptor MoAb (αIR-3) or mouse control IgG (MOPC21) was added at culture medium at final concentration of 15 μg/mL. IGF-II was added 2 hours later after the initiation of cultures containing αIR-3 or control IgG.

**Preparation of bone marrow (BM) stromal cells.** BM specimens were obtained from 4 patients with multiple myeloma after informed consent was given. Mononuclear cells separated by Ficoll-Hypaque density sedimentation were used to prepare BM stromal cells. Mononuclear cells were suspended in 10 mL of RPMI 1640 medium containing 10% FCS. Cells were incubated at 37°C in a CO2 incubator. After 3 or 4 days, 5 mL of fresh medium was added. When an adherent cell layer had developed, the cells were harvested in PBS containing 0.25% trypsin and 0.02% EDTA. BM stromal cells prepared individually from 4 patients with multiple myeloma were designated as Str-1, Str-2, Str-3, and Str-4.

**RESULTS**

Treatment of OPM-2 cells with CSP led to an increase in their adhesion to HUVECs. To determine the effect of CSP on the adhesion of OPM-2 cells to HUVECs, OPM-2 cells were cultured with or without CSP (200 μg/mL) for 48 hours and then cocultured with HUVECs. In the absence of CSP treatment, 17.8% of OPM-2 cells adhered to HUVECs (Fig 1). The preincubation of OPM-2 cells with CSP led to a significant increase in adhesion of OPM-2 cells, and 24.6% of OPM-2 cells were found to adhere to HUVECs (Fig 1). Although CSP treatment is known to increase the high-mannose-type glycoproteins on the cell surface, the addition of mannosidase did not abrogate the CSP-induced increase in adhesion of OPM-2 cells to HUVECs (data not shown). We next examined the effect of CSP on surface expression of adhesion molecules, which were known to express on myeloma cells. Flow cytometry analysis showed that CD44 and CD49d were expressed on OPM-2 cells at a high level, and CD54 at a moderate level, whereas little or no expression of CD11a, CD18, CD49e, or LAM-1 was observed on the cells (Table 1). The surface expression of the adhesion molecules were not influenced by the treatment of CSP (Table 1). These results indicated that CSP did not affect the expression of the well-known adhesion molecules and suggested that CSP might influence the function of adhesion molecules by altering their oligosaccharide structures.

**CSP induced alteration in oligosaccharide structure of GP220.** To detect glycoproteins whose oligosaccharides were altered to high-mannose type by the treatment with CSP, the cell lysates prepared from CSP-treated and CSP-untreated OPM-2 cells were subjected to lectin-binding analysis using RCA and Con-A, which recognize galactose and mannose, respectively. The efficient effect of CSP on glycoprotein should result in its decreased reactivity with RCA and also in its increased reactivity with Con-A. As shown in Fig 2, CSP induced an increased reactivity with Con-A in proteins particularly at molecular weight of 220 and 150 kD under nonreducing condition and a decreased reactivity with RCA in proteins, particularly at molecular weight of
Table 1. Effect of CSP Treatment on Surface Expression of Adhesion Molecules

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CD11a</th>
<th>CD18</th>
<th>CD44</th>
<th>CD54</th>
<th>CD49d</th>
<th>CD49e</th>
<th>LAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>93.7</td>
<td>31.1</td>
<td>96.8</td>
<td>0</td>
<td>4.6</td>
</tr>
<tr>
<td>CSP</td>
<td>0</td>
<td>0</td>
<td>95.5</td>
<td>28.7</td>
<td>96.6</td>
<td>0</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Abbreviation: CSP, castanospermine.

220 kD. Thus, the 220-kD glycoprotein (GP220) was one of the major Con-A- and RCA-reactive glycoproteins, and the treatment of CSP increased Con-A affinity and decreased RCA affinity in GP220, indicating that the oligosaccharides of GP220 were altered to high-mannose type by CSP. Furthermore, it was noted that GP220 was detectable in cell surface proteins that were labeled with sulfo-NHS-biotin (data not shown).

Involvement of GP220 in adhesion of OPM-2 to HUVECs. To determine if GP220 was involved in the adhesion of OPM-2 cells to HUVECs, GP220-enriched glycoproteins were prepared from lysate of OPM-2 cells by RCA-agarose column. The glycoproteins were subjected to SDS-PAGE under nonreducing or reducing conditions and blotted to PVDF membrane. After staining with RCA and Coomassie Blue R-250, GP220 was found to be at molecular weight of 220 kD as a major glycoprotein under nonreducing condition, and under reducing condition, GP220 was found to shift at molecular weight of 240 kD (Fig 3A). Cell surface glycoproteins of HUVECs were then labeled by biotin, and biotin-labeled glycoproteins were incubated with the PVDF membrane to which GP220-enriched glycoproteins were blotted. As shown in Fig 3B, biotinylated glycoproteins at the plasma membrane of HUVECs bound to GP220 of OPM-2 cells under nonreducing condition. The binding of HUVEC-glycoproteins to GP220 was inhibited by the addition of 10 mmol/L M6P but not by that of 20 mmol/L EDTA (Fig 3C).

Molecular characterization of GP220. The purification of GP220 from OPM-2 cells was performed by RCA-affinity chromatography, followed by preparative SDS-PAGE. Approximately 50 μg of GP220 was isolated from Coomassie Blue-stained gels. The pooled GP220 was then digested with endoproteinase Lys-C. This digestion of GP220 resulted in development of approximately 70-kD band (Fig 4A), and this protein was subjected to automated gas-phase peptide sequence. Seven amino acid sequence in NH₂-terminus of the protein was Thr-Gly-Pro-Val-Val-Glu-Asp (TGPVVED). A search of NBRF protein identification resource revealed that a seven amino acid sequence was detectable in human CIM6P/IGF-II receptor. To determine whether or not GP220 was CIM6P/IGF-II receptor, the GP220 fraction was subjected to immunoblotting with a specific antibody against CIM6P/IGF-II receptor. The anti-CIM6P/IGF-II receptor antibody was found to recognize GP220 (Fig 4B). Under reducing condition, furthermore, the antibody was reactive with 240-kD protein expressed on OPM-2 cells.

CIM6P/IGF-II receptor (GP220) mediates adhesion via its interaction with M6P. To confirm that GP220 (CIM6P/IGF-II receptor) mediated the adhesion of OPM-2 cells to HUVECs via M6P moieties of glycoproteins expressed on HUVECs, we examined the inhibitory effect of M6P on the adhesion. As shown in Fig 5A, the adhesion of OPM-2 cells to HUVECs was inhibited by M6P in a dose-dependent manner. By contrast, the adhesion of OPM-2 cells to HUVECs was not inhibited by mannose or galactose (Fig 5B). Furthermore, the inhibitory effect of M6P on the adhesion to HUVECs was also observed in other types of human myeloma cell lines, RPMI 8226 and U266 (Fig 6A). The treatment with M6P decreased binding of RPMI 8226 cells to HUVECs by 25% (39.9% to 29.8%) and decreased binding of U266 cells to HUVECs by 30% (9.7% to 6.8%).

To further determine if the CIM6P/IGF-II receptor was involved in the adhesion of myeloma cells to BM stromal cells, we investigated the effect of M6P on the adhesion of human myeloma cell lines (OPM-2, RPMI 8226, and U266) to BM stromal cells, named as Str-1, -2, -3, and -4, which were prepared from 4 patients with multiple myeloma. As shown in Fig 6B, the addition of M6P resulted in a significant decrease in the adhesion of OPM-2 cells to each type of BM stromal cells and that of RPMI 8226 cells to three types of BM stromal cells (Str-1, -2, and -3), suggesting the involvement of CIM6P/IGF-II receptor in binding myeloma cells to stromal cells.
In addition to the oligosaccharide structures of GP220, it was also speculated that CSP might have an effect on the expression of GP220 (CIM6P/IGF-II receptor). We therefore labeled OPM-2 cells with sulfo-NHS-biotin before and after treatment with CSP, and the lysates prepared from the cells were immunoprecipitated with anti-CIM6P/IGF-II receptor antibody. As shown in Fig 7, the expression of GP220 (CIM6P/IGF-II receptor) was slightly enhanced by treatment with CSP.

**CIM6P/IGF-II receptor (GP220) mediates proliferation signal of IGF-II.** Because IGF-II was known to promote growth of various types of cells, we examined the effect of IGF-II on proliferation of OPM-2 cells. Proliferation of OPM-2 cells was assayed with MTT colorimetric method.
findings have been reported previously in human myeloma cells.

The CIM6P receptor is known to participate in the targeting of lysosomal enzymes to the lysosome by binding to M6P residues that are present on N-linked oligosaccharides of the hydrolytic enzymes. On the other hand, IGF-II receptor was identified as CIM6P receptor by deduction from the complementary DNA sequence encoding the receptor. IGFI and M6P bind to separate sites on the receptors. It has been reported that about 10% of CIM6P/IGF-II receptor are present at cell surface where the receptor functions to bind and internalize exogenous lysosomal enzymes and IGF-II. This receptor has been also been shown to mediate IGF-II-induced signal transduction that leads to cell growth, incorporation of sulfate into cartilage cells, and modulation of myoblast differentiation. In addition to these functions, it has been further demonstrated that CIM6P/IGF-II receptor on the cell surface contributes to activate the transforming growth factor-β (TGF-β) by binding M6P moieties of TGF-

DISCUSSION

The present study was carried out to identify adhesion molecules that were involved in adhesion of a human myeloma cell line (OPM-2) to HUVECs. We have found that CIM6P/IGF-II receptor expressed on OPM-2 cells has an activity in supporting adhesion of the cells to HUVECs. It was also demonstrated that CIM6P/IGF-II receptor was capable of mediating proliferation signal into OPM-2 cells after binding of IGF-II. To our knowledge, none of these
A number of adhesion molecules or adhesive activities have been found to be involved in the interaction of hematopoietic cells with BM stromal cells and extracellular matrix molecules. In myeloma cells that are predominantly accumulated in bone marrow, VLA-4/fibronectin, VLA-4/VCAM-1, and CD44/hyaluronate have been reported to contribute to adhesion. Recent reports suggest that cell-cell adhesion is not mediated by a single receptor/ligand pair but rather by multiple interactions consisting of several different receptor/ligand pairs. The multiple interactions are suggested to be required for stable adhesion and for the specificity of adhesive interactions observed in vivo. Furthermore, it has been proposed that the engagement of one receptor with its ligand may trigger enhanced adhesion through another receptor/ligand pair. In this study, we have found that CIM6P/IGF-II receptor of OPM-2 cells has an adhesion-supporting activity via its interaction with M6P moieties expressed on HUVECs. We have further found that the M6P-mediated adhesion was observed in the interaction between myeloma cell lines and BM stromal cells. These findings suggest that a new receptor/ligand pair, CIM6P/IGF-II receptor/M6P moiety, may be involved in adhesion of myeloma cells to stromal cells.

This study demonstrated that IGF-II was a growth factor for OPM-2 cells. IGF-II has a structure highly homologous to insulin that regulates cell growth and differentiation in addition to glycogenolysis. Furthermore, IGF-II is functionally and structurally related to IGF-I, and both factors have been shown to act in an autocrine/paracrine fashion in stimulating cell growth and differentiation. A number of previous reports have indicated that most of the metabolic and mitogenic effects of IGF-II are mediated by IGF-I receptor, which contains an intrinsic ligand-activated tyrosine kinase. In some cases, however, IGF-II has been shown to exert its function through binding to the monomeric IGF-II receptor that lacks tyrosine kinase activity in its cytoplasmic domain but is coupled to a Gα12 guanine nucleotide-binding protein. The results of this study showing that CIM6P/IGF-II receptor mediates adhesion and proliferation of OPM-2 cells provided additional roles of the receptor in adhesion and growth of myeloma cells.

β. TGF-β is secreted from cells as a latent complex containing phosphomannosyl residues. On the cell surface, the proteolytic activation of TGF-β is facilitated by its finding to CIM6P/IGF-II receptor. Thus, CIM6P/IGF-II receptor has been shown to be multifunctional binding protein. The...
protein. 44,45 For example, proliferation of tumor cells such as K562 were stimulated by IGF-II via the IGF-II receptor. 46 In the case of B cells, IGF-I receptor is well known to support proliferation of pro-B cells and myeloma cells. 47,48 However, the role of CIM6P/IGF-II receptor in proliferation and differentiation of B cells has not been characterized in detail. In this study, we first demonstrated that IGF-II stimulates the proliferation of OPM-2 cells via CIM6P/IGF-II receptor. Furthermore, our preliminary experiments indicate that IGF-II is produced by BM stromal cells prepared from patients with multiple myeloma as well as healthy adults. These results suggest that CIM6P/IGF-II receptor may play a role in growth regulation of plasma cell.

In the past decades, the high activities of lysosomal enzymes, including acid phosphatase, are known to be of great value in diagnosis of advanced multiple myeloma. 50 How- ever, the mechanism responsible for high expression of lysosomal enzymes in myeloma cells remains to be unclear. Because CIM6P/IGF-II receptor is a carrier protein of lysosomal enzymes, it is possible that the receptor has a role in high expression of lysosomal enzymes in advanced myeloma cells. In light of data presented here, furthermore, CIM6P/IGF-II receptor may contribute to abnormal growth and localization of myeloma cells. Because recent reports have suggested that disruption of adherence of myeloma cells to BM stromal cells may affect not only growth of myeloma cells, but also regrowth of the cells in bone marrow after peripheral blood stem cell transplantation, 51,52 clear information regarding the adhesion molecules, including CIM6P/IGF-II receptor, will be important to construct novel therapeutic strategies for the treatment of patients with multiple myeloma. Further studies will be necessary to clarify the biological and clinical significance of CIM6P/IGF-II receptor in multiple myeloma.

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