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Ribosomal protein metallopanstimulin-1 impairs multiple myeloma CAG cells growth and inhibits fibroblast growth factor receptor 3

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Abstract

We demonstrated that metallopanstimulin-1 (MPS-1, RPS27) inhibited growth of tumors formed by head and neck squamous cell carcinoma cells and reduced paxillin gene expression. The present study examined whether and how MPS-1 affects another type of cancer, multiple myeloma (CAG). Enhanced expression of MPS-1 dramatically inhibited CAG *in vitro* and *in vivo*. Overexpression of MPS-1 resulted in decreased fibroblast growth factor (FGF) receptor 3 and impaired endogenous MAPK/ErK signaling. MAPK/ErK signaling was not stimulated by adding recombinant FGF2 to myeloma cells overexpressing MPS-1. These data suggest that MPS-1 suppresses CAG growth and that weakened FGF signaling may contribute to this effect.

Keywords

metallopantstimulin-1; RPS27; FGFR3; multiple myeloma

1. Introduction

Ample evidence has emerged demonstrating that functions of ribosomal proteins (r-proteins) are not limited to protein biosynthesis in the ribosome. R-proteins have extensive and complicated extraribosomal functions involved in DNA repair, apoptosis, and even tumor development [1–8]. For example, in *Drosophila*, r-protein S3 (rpS3) has DNase activity and DNA deoxyribophosphodiesterase activity that is needed to repair DNA damage induced by oxidative and ionizing radiation [1, 2, 3]. Some other r-proteins, such as S29, S27L, S13, and L3, can either promote or suppress apoptosis in different types of cells [4, 5, 6], and increased expression of rpS19 improves erythroid development in cells originating from rpS19-deficient Diamond Blackfan anemia [7].

Importantly, some r-proteins may have a close relation to oncogenesis. Enhanced expression of RPS3a appeared to induce transformation of NIH 3T3 cells and formation of tumors in nude mice [8]. Our previous studies [9] clearly demonstrated that metallopantstimulin-1

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Conflict of Interest

The authors do not have any conflicts of interest to report for this manuscript.

(MPS-1), human RPS27 encoded by a TGF- β 1-inducible gene [10], dramatically inhibited growth of human head and neck squamous cell carcinoma (HNSCC) UMSCC-1 cells both *in vitro* and *in vivo*. Furthermore, MPS-1 suppressed paxillin gene expression, inhibited tumor cell proliferation, and reduced tumor angiogenesis, which are factors thought to be important to growth and tumorigenicity of UMSCC-1 cells.

Our previous findings with HNSCC cells and MPS-1 showed that the gene mapped to chromosome 5q. Upon review of other diseases in which this portion of the genome may be significant, we appreciated that RPS14 is a putative tumor suppressor gene and a candidate for gene deletion in 5q-myelodysplastic syndrome. Therefore, we examined the effects of MPS-1 protein expression on the biological behaviors of a mesenchymal B cell malignancy, multiple myeloma, which differs in its origins from an epithelially derived HNSCC [11]. We are interested in exploring whether the effect and mechanism of MPS-1 suppression is the same or different across malignancy cell types.

Our data indicate that enhanced expression of MPS-1 protein in myeloma cells (CAG) dramatically inhibited cell growth *in vitro* and *in vivo*. It also resulted in reduced expression of fibroblast growth factor receptor 3 (FGFR3) rather than that of paxillin, as in HNSCC cells, and suppressed the FGF signaling pathway, which may greatly contribute to the inhibitory effects on myeloma cell growth.

2. Materials and Methods

2.1. Cell line and cell culture

Cells of human multiple myeloma cell line CAG [12] (kindly provided by Ralph D. Sanderson, PhD, University of Alabama at Birmingham, Birmingham, AL), were cultured in RPMI 1640 medium containing L-glutamine (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin sulfate, and were maintained at 37°C in an incubator containing 5% CO₂.

2.2. Plasmid construction and transfection

cDNA for MPS-1 tagged with His(6) at the C-terminal was cloned into pIRES2-EGFP expression vector, which is the same vector used in our previous research on human HNSCC cells [9]. CAG cells were transfected with 10 μ g of either empty vector or pIRES2-EGFP/MPS-1, using Lipofectamine 2000 (Invitrogen) and Opti-MEM I (Invitrogen) according to the manufacturer's instructions. Successfully transfected cells were sorted by flow cytometry using EGFP as the selection marker. Before *in vitro* and *in vivo* experiments, the percentage of EGFP-positive cells was greater than 90%.

2.3. Protein isolation and Western blotting

Cells were pelleted by centrifugation, rinsed with ice-cold PBS, and lysed at room temperature in 200 μ l of M-PERT[™] tissue protein extraction reagent (Pierce, Rockford, IL) and then with Halt[™] Protease Inhibitor Cocktail (Pierce). Lysates were centrifuged at 14,000g for 15 min at room temperature. Lysates (20 μ l) were loaded onto NuPAGE 4–12% Bis-Tri gels (Invitrogen) for electrophoresis and transferred to a nitrocellulose membrane. Membranes were probed with one of the following antibodies: monoclonal mouse antibodies against His(6) (1:1,000) (GenScript, Piscataway, NJ), FGFR3 (B-9) (1:200) (Santa Cruz Biotechnology, Santa Cruz, CA), or beta-actin (C4) (1:1,000) (Santa Cruz Biotechnology); or monoclonal rabbit antibodies against pp44/42 (1:1,000) (Cell Signaling Technology, Danvers, MA) or p44/42 (1:1,000) (Cell Signaling Technology). The blot was incubated with a horseradish peroxidase (HRP)-conjugated antibody—either rabbit anti-mouse IgG or goat anti-rabbit IgG (both from AnaSpec, San Jose, CA). Proteins were visualized by using

ECL Western Blotting Substrate kit (Pierce) according to the manufacturer's instructions. Protein levels were semi-quantitatively measured and normalized using NIH software Image J (National Institutes of Health, Bethesda, MD).

2.4. Dot blotting

To determine whether MPS-1 was secreted into the extracellular space, conditioned media from CAG cells carrying pIRES2-EGFP/MPS-1 or empty vector were analyzed. Cells (5×10^5 cells/ml) were cultured for 48 h; media were then collected, and aliquots (200 μ l) were dot-blotted onto a nitrocellulose membrane. The blot was probed with monoclonal mouse antibody against His(6) (1:1,000 dilution), followed by biotin-conjugated goat anti-mouse IgG secondary antibody (Vector Laboratories, Burlingame, CA). The protein dots were visualized by using ECL Western Blotting Substrate kit (Pierce).

2.5. Cell fractionation

To examine the cellular localization of MPS-1 in CAG cells overexpressing MPS-1, subcellular fractions were prepared with a Nuclear/Cytosol Fractionation Kit (BioVision, Mountain View, CA), which ensures little or no cross-contamination (<http://www.biovision.com/pdf/K266.pdf>).

2.6. Analysis of FGF signaling

To investigate the change of endogenous FGF signaling, cells (3.0×10^6) in the log phase of growth in medium with 10% fetal bovine serum were harvested. In addition, to examine the change of FGF signaling in the cells exposed to the exogenous FGF, cells (3.0×10^6) in the log phase of growth were serum-starved overnight and then treated with either 5 ng/ml or 100 ng/ml of FGF basic (R&D Systems, Minneapolis, MN) for 1 h at 37°C and then harvested. Cells were rinsed with ice-cold PBS and lysed at room temperature, as described above. Changes in FGF signaling were determined by using Western blotting (described above) to analyze levels of phosphorylated MAPK/ErK.

2.7. Tumor cell proliferation assay

To assess tumor cell proliferation *in vitro*, the transfected cells were seeded in 12-well plates at a density of 1×10^5 cells/ml with complete RPMI 1640 medium in triplicate. Plates were incubated at 37°C in 5% CO₂ for 0, 1, 2, 3, or 4 days. Daily, cells were collected and counted, and cell densities were calculated. To investigate whether biosynthesized MPS-1 protein inhibits CAG cell growth, the parental cells were seeded in 12-well plates at a density of 1×10^5 cells/ml with complete RPMI 1640 medium. In the treatment groups, MPS-1 protein produced by Promab (ProMab Biotechnologies, Richmond, CA) was added to the well at either low concentration (0.1 μ g/ml) or high concentration (0.5 μ g/ml). In the control group, the inactivated MPS-1 protein, heated at 100°C for 10 minutes, was added.

2.8. Tumor growth *in vivo*

CAG cells carrying either pIRES2-EGFP/MPS-1 or empty vector were harvested, resuspended in ice-cold PBS (100 μ l), and subcutaneously injected into the left flank of a 5-week-old male athymic nude mouse (1×10^6 cells/mouse) (Harlan Sprague Dawley, Indianapolis, IN). Tumor growth was monitored weekly by measuring tumor size, and whole-mouse tumor burden was determined by using enzyme-linked immunosorbent assays (ELISAs) to measure serum levels of IgG κ -light chain. All samples were analyzed in duplicate and simultaneously. Samples were diluted to concentrations within the linear range of the standard curve (15–1,000 ng/ml). Upon sacrifice, about 5 weeks after injection of cells, tumors were harvested and weighed. Tumor tissue was then divided, and a portion was snap-frozen in liquid nitrogen and then stored at –80°C. The remaining portion of tumor

tissue was fixed in 10% formalin (pH 7.0), embedded in paraffin, and stained with hematoxylin and eosin for histology. All the procedures were approved by the Institutional Animal Care and Use Committee of the University of Arkansas for Medical Sciences and conformed to guidelines established by the National Institutes of Health.

2.9. Tumor TdT-mediated dUTP-biotin nick end labeling (TUNEL) staining

To detect apoptosis on tumor tissue sections, Dead-End Colorimetric TUNEL System (Promega, Madison, WI) was used according to the manufacturer's manual. Briefly, after being deparaffinized, rehydrated, and permeabilized by proteinase K, the tumor sections were incubated with TdT reaction mix (60 min at 37°C) and then with Streptavidin HRP solution (1:500; 30 min at room temperature); staining was visualized by incubating the sections with 3,3'-diaminobenzidine solution.

2.10. Gene array analysis

Total RNA was extracted from cells by using Trizol Reagent (Invitrogen). Gene expression profiling was performed with the GeneChip Human Genome U133 Plus 2.0 microarray platform (Affymetrix, Santa Clara, CA) [13]. Determination of mRNA levels was performed with Affymetrix microarray suite GCOS1.1 software.

2.11. Statistical analyses

All data are expressed as mean $\pm$ standard deviation, and the differences between the control group and MPS-1 group were calculated with the Student's *t* test. Statistical significance was set as $p < 0.05$.

3. Results

3.1. Overexpressed MPS-1 was detected in transfected CAG myeloma cells and in conditioned medium

To express high levels of MPS-1 protein in multiple myeloma CAG cells, the cells were transfected with a plasmid that contained the cDNA for MPS-1 tagged with His(6) at the C-terminal. Western blotting the cell lysates confirmed that MPS-1 protein was highly expressed in cells transfected with the plasmid encoding the His-tagged protein but not in control cells transfected with empty vector (Fig. 1A). Western blotting also revealed that His-tagged MPS-1 was present in both the cytosolic fraction and the nuclear fraction (Fig. 1B) [14]. For quantitation, MPS-1 levels were normalized to those of cytoplasmic β -actin and nuclear β -actin, which were used as loading controls. Furthermore, dot blotting analysis of conditioned media from the cultured cells showed that His-tagged MPS-1 was secreted into the medium by CAG/MPS-1 cells (Fig. 1C). These findings are consistent with our previous findings in human HNSCC cells [9].

3.2. Enhanced expression of MPS-1 reduced FGFR3 expression and impaired MAPK/ErK signaling

Because FGFR3, a tyrosine kinase receptor and transmitter of MAPK signaling, plays an important role in proliferation of myeloma cells [15], we next examined effects of MPS-1 overexpression on FGFR3 signaling. Western blotting showed that FGFR3 levels in CAG/MPS-1 myeloma cells were approximately one-third of FGFR3 levels in control cells (Fig. 2A). Effects of MPS-1 overexpression on signaling downstream of FGFR3 were then analyzed, using Western blots to examine the levels of phosphorylated ErK 44/42 (pp44/42). Under the normal cell culture conditions with 10% fetal bovine serum, Western blots of cell extracts revealed that CAG cells expressing MPS-1 exhibit decreased levels of pp44/42 as compared with the control (Fig. 2B). In addition, we investigated activation of the MAPK

signaling pathway in CAG cells by exogenous FGF2, known as ligand for FGFR3. Adding recombinant FGF2 (5 ng/ml and 100 ng/ml) to the cells, which were starved in serum-free medium overnight to eliminate endogenous MAPK signaling activity, resulted in significantly increased pp44/42 levels in the control CAG cells as compared with CAG/MPS-1 cells (Fig. 2C). These findings indicate that MPS-1 downregulates FGFR3 expression in myeloma CAG cells and weaken FGFR3-related functions, such as growth factor signaling transduction.

3.3. Both increased expression of MPS-1 and biosynthesized MPS-1 protein inhibited cell proliferation *in vitro*

In vitro cell proliferation assays were used to investigate the effects of increased expression of MPS-1 on the growth rate of CAG myeloma cells. In CAG/MPS-1 cells, growth was significantly slower than in control cells ($p < 0.01$) (Fig 3A). Furthermore, addition of either high or low concentration of biosynthesized MPS-1 protein inhibited growth of cultured CAG cells (control vs. high concentration: $p=0.04$; control vs. low concentration: $p=0.005$) (Fig 3B).

3.4. Enhanced expression of MPS-1 inhibited tumor growth *in vivo*

Effects of MPS-1 overexpression on tumor growth in mice were examined. Mice were injected with CAG cells transfected with either empty vector or pIRES2-EGFP/MPS-1, and resultant tumors were compared. Expression of MPS-1 greatly inhibited tumor growth ($p = 0.004$), based on monitoring levels of IgG κ -light chain in mouse serum as a measure of myeloma tumor burden (Fig. 4A). Upon sacrifice (week 5), the mean weight of the primary tumors formed by CAG/MPS-1 cells was 0.12 ± 0.05 g ($n = 6$) and that of primary tumors formed by control cells was 1.98 ± 1.43 g ($n = 6$) ($p = 0.009$) (Fig. 4B). These findings were confirmed in a subsequent experiment, which showed similar growth inhibition of tumors formed by CAG/MPS-1 cells (CAG/MPS-1, 0.22 ± 0.26 g; control, 3.66 ± 1.63 g; $n = 3$ for each group; $p = 0.01$) (Fig. 4C).

3.5. Overexpression of MPS-1 did not induce tumor cell apoptosis

To investigate whether tumor growth inhibition was due to apoptosis, the tumors were sectioned and subjected to TUNEL assays. Results of TUNEL assays for tumors that originated from CAG/MPS-1 cells were similar to those from control cells; in both cases, few cells were TUNEL-positive (Fig. 5), which indicates that apoptosis is not the source of the observed reduction in growth of tumors formed by CAG/MPS-1 cells.

3.6. Gene expression profiling

Gene expression profiling was performed to replicate in CAG myeloma cells the work that was done with MPS-1 overexpression in HNSCC [9]. This was also done to identify other potential pathways of putative mechanisms for MPS-1 activity in CAG myeloma cells that could be explored in the future. Gene expression profiling was performed on total RNA extracted from CAG/MPS-1 cells and from control CAG cells. mRNA levels of genes identified by probe sets of the GeneChip Human Genome U133 Plus 2.0 array were determined and compared (Table 1).

4. Discussion

Our previous study of HNSCC cells showed that MPS-1 protein was present in the cytosol, nucleus, and extracellular space, and that high levels of MPS-1 strongly inhibited cell growth [9]. The present study in multiple myeloma CAG cells revealed similar findings. MPS-1 was detected in cytosolic and nuclear fractions and in conditioned media of cells

overexpressing the protein. High levels of MPS-1 not only dramatically inhibited CAG cell proliferation in culture but also strongly suppressed CAG tumor formation and development in mice. Despite the diverse origins of multiple myeloma (hematopoietic) and HNSCC (epithelial), overexpression of MPS-1 elicited similar responses in both types of cancer cells, which indicates that MPS-1 is a novel and significant tumor suppressor for different types of tumors. Therefore, MPS-1 might be a new, promising molecular target and/or biomedical drug in fighting cancer.

Although MPS-1 has inhibitory effects on both HNSCC and myeloma cells, the mechanisms involved appear to be different in the two types of tumors. Our previous study showed that paxillin expression is suppressed in MPS-1-transfected HNSCC cells [9]; however, paxillin is expressed at low levels in CAG myeloma cells, and its expression is not changed by MPS-1 transfection (data not shown). Because the FGF signaling pathway has been demonstrated to play an inhibitory role in growth of CAG myeloma cells, effects of MPS-1 on this pathway were examined. The results support the following observations about the molecular functions of MPS-1 in CAG cells: (1) MPS-1 reduces FGFR3 expression, as evidenced by Western blotting that showed significantly less FGFR3 protein in CAG/MPS-1 cells than in control cells; (2) MPS-1 overexpression impedes endogenous FGF signaling in CAG myeloma cells; and (3) CAG cells overexpressing MPS-1 have diminished reactions to exogenous FGF2 stimulation with blocked receptor signaling. These findings indicate that MPS-1 inhibits robust growth of CAG cells, possibly by directly targeting FGFR3 and its corresponding MAPK/ErK signaling pathway.

A member of a family of transmembrane receptors for tyrosine kinases, FGFR3 plays a key role in regulating cell growth and proliferation of different types of cancers, including multiple myeloma. Activated FGFR3 contributes to tumor progression in multiple myeloma [15]. Fifteen percent of patients with myeloma have the t(4;14) translocation that leads to overexpression of FGFR3, and many of these patients with t(4;14) translocations have deletions involving the FGFR3 gene.

Targeting FGFR3 to treat multiple myeloma has been proposed and tested. The neoplastic potential of FGFR3 was directly studied by transplanting animal models with FGFR3-TD-expressing bone marrow, which resulted in the development of B-cell neoplasms [16]. Two tyrosine kinase inhibitors, SU5402 and PD173074, inhibit growth and reduce survival of multiple myeloma cell lines KMS-11 and OPM-2 by blocking FGFR3 function [17]. R3Mab, an antibody against wild-type FGFR3 and its various mutants, binds the receptor and blocks its signaling, which results in growth inhibition of multiple myeloma xenografts with t(4;14) translocations and with high expression levels of FGFR3 [18]. These observations of FGFR3 in myeloma cells are supported by our findings that MPS-1-transfected CAG myeloma cells have decreased levels of FGFR3 and that these cells form tumors with dramatically inhibited growth and proliferation. At this time, however, little other evidence demonstrates that FGFR3/FGF signaling plays a major role in multiple myeloma.

Two mechanisms are known to be involved in the suppression of multiple myeloma that results from diminished FGFR3 signaling. First, decreased FGFR3 signaling results in apoptosis of myeloma cells. Decreasing FGFR3 expression, by using short hairpin RNAs specifically targeting FGFR3 mRNA, induced apoptosis in human myeloma cell lines KMS-11, OPM-2, and NCI-H929 [19]. Using a specific inhibitor, PD173074, to inhibit FGFR3 activity induced differentiation and apoptosis of myeloma cells, and it delayed tumor progression and prolonged survival of mice with myeloma cells that expressed high levels of FGFR3 [20]. In our studies, however, the reduced levels of FGFR3 that resulted from MPS-1 overexpression did not increase apoptosis in CAG myeloma cells.

In the second mechanism, reduced signaling through the FGFR3 receptor contributes to inhibited growth of myeloma cells. Blocking FGFR3 by using specific tyrosine kinase inhibitors (SU5402 and PD173074) resulted in diminished FGFR3 signaling, indicated by decreased levels of phosphorylated MAPK/ErK pp44/42, and suppressed growth of KMS-11 and OPM-2 myeloma cells [17]. Our findings are consistent with this second mechanism. Overexpression of MPS-1 reduced FGFR3 protein levels in CAG myeloma cells and interfered with its downstream signaling, as evidenced by reduced phosphorylation of p44/42, which has been shown to be related closely to inhibition of CAG myeloma cell growth [21].

Although the mechanism for MPS-1 downregulation of FGFR3 protein levels is not yet known, FGFR3 gene transcription was not affected by MPS-1 overexpression, based on gene expression profiling that showed FGFR3 mRNA levels are the same in cells overexpressing MPS-1 as in control cells (not shown). Therefore, MPS-1 probably decreases FGFR3 levels by effects on translation. Because MPS-1 is a ribosomal protein, it is likely to have global effects on ribosomal function and translation, rather than to target a single signaling pathway. Therefore, it is unclear whether forced overexpression of MPS-1 has an isolated effect on FGFR/FGF pathways.

In conclusion, this study showed that ribosomal protein MPS-1 (RPS27) dramatically inhibited growth of multiple myeloma cells via mechanisms different from those observed in HNSCC cells. MPS-1 strongly downregulated FGFR3 protein levels and reduced its signal transduction. Growth of CAG myeloma cells that depend on FGFR3 signaling for proliferation and development was, therefore, dramatically inhibited. MPS-1 potentially could be used therapeutically to suppress multiple myeloma, which is a novel use for a ribosomal protein.

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Fig. 1. Exogenous MPS-1 protein was present in CAG/MPS-1 cells and in conditioned medium (a) MPS-1 was present in lysates from cells transfected with MPS-1 (M) but not in control cells transfected with empty vector (C). Western blots were probed with a monoclonal anti-His antibody that recognizes His-tagged MPS-1 protein (~10 kDa). β -actin was used as the protein loading control. (b) MPS-1 was present in the cytosol and the nucleus. (c) Dot blotting with the anti-His antibody detected His-tagged MPS-1 in the conditioned medium from CAG/MPS-1 cells (M) but not from control cells (C).

Fig. 2. MPS-1 reduced FGFR3 protein expression and blocked MAPK/ErK signaling

(a) Cell lysates were analyzed with Western blotting, which showed lower levels of FGFR3 protein in MPS-1-transfected cells (M) than in control cells (C). (b) When cultured in standard rich medium, CAG/MPS-1 cells (M) had diminished pp44/42, which indicates MAPK/ErK signaling activity, while control cells (C) had activated signaling. (c) Serum-starved cells were treated with exogenous recombinant FGF2 at low concentration (5 ng/ml) and high concentration (100 ng/ml). The MAPK/ErK signaling pathway, indicated by pp44/42 level, was not activated in CAG/MPS-1 cells (M) but was activated in control cells (C). (pp44/42: phosphorylated ErK 44/42 protein; p44/42: total ErK 44/42 protein).

Fig. 3. Both overexpressed and exogenous MPS-1 protein inhibited growth of CAG cells *in vitro* (a) Control cells (◆) or MPS-1-transfected cells (■) were plated in 12-well plates (1×10^5 cells/well) and were counted on 4 consecutive days (*, $p < 0.01$; bars, SD). (b) CAG cells were treated with either inactivated MPS-1 protein (■), low-concentration (0.1 $\mu\text{g/ml}$) MPS-1 protein (◆), or high-concentration (0.1 $\mu\text{g/ml}$) MPS-1 protein (●) (*, $p < 0.04$; bars, SD).

Fig. 4. High levels of MPS-1 protein inhibited growth of CAG cells *in vivo*

(a) Cells were injected subcutaneously into the left flanks of male nude mice (1×10^6 cells/mouse). Tumor burden was determined by measuring IgG κ -light chain in mouse serum every other week (*, $p = 0.004$, control *versus* MPS-1; bars, SD). (b) Tumors (indicated by arrow) were removed from mice upon sacrifice (week 5) and were weighed (*, $p = 0.009$; columns, mean tumor weight; bars, SD). (c) Subsequent mouse experiment had similar results, as shown by the photograph and tumor weights (*, $p = 0.01$; columns, mean tumor weight; bars, SD).

Fig. 5. Inhibitory effects of MPS-1 on growth of CAG cells were not due to apoptosis
Apoptosis was determined by TUNEL assays of tumor tissues (magnification, 100×). Positive staining, indicating apoptosis, was evident only in few cells from tumors formed from either CAG/MPS-1 cells or CAG control cells. The positive control slide was treated with DNase I before staining.

Table 1

Results of gene expression profiling of CAG with and without MPS-1 over expression

Upregulated		Downregulated	
Gene name	Fold change*	Gene name	Fold change*
Interleukin 32	134	Uncharacterized gastric protein ZA31P	>1,000
Early growth response 1	98	CD9 antigen (p24)	100
B lymphoid tyrosine kinase	51	Hairy/enhancer-of-split related with YRPW motif 2	100
Sequestosome 1	42	Uncharacterized gastric protein YC12P	50
Heat shock 70 kDa protein 1A	40	Oxysterol binding protein-like 1A	33
Rho GTPase activating protein 15	21	Myristoylated alanine-rich protein kinase C substrate	33
Insulin receptor substrate 2	18	Synuclein, alpha interacting protein (synphilin)	25
Early growth response 1	16	Janus kinase 3 (a protein tyrosine kinase, leukocyte)	25
Sphingomyelin phosphodiesterase, acid-like 3A	16	FLJ42957 protein	20
Ephrin-A1	15		
Tumor necrosis factor receptor superfamily, member 25	14		
Mannosidase, alpha, class 1C, member 1	13		
Short coiled-coil protein	12		
Heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1	12		

* Fold change in mRNA level in CAG/MPS-1 cells as compared to CAG control cells.