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HuBMSC-MCP, a novel member of mitochondrial carrier superfamily, enhances dendritic cell endocytosis

Baomei Wang,[a] Nan Li,[a] Lili Sui,[a] Yanfeng Wu,[a] Xiaojian Wang,[b] Qingqing Wang,[b] Dajing Xia,[b] Tao Wan,[a] and Xuetao Cao[a,b,*]

[a] *Institute of Immunology, Second Military Medical University, Shanghai 200433, P.R. China*
[b] *Institute of Immunology, Zhejiang University, Hangzhou 310031, Zhejiang, P.R. China*

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Abstract

A novel member of mitochondrial carrier superfamily has been identified from human bone marrow stromal cells (BMSC) and designated as human BMSC-derived mitochondrial carrier protein (HuBMSC-MCP). It encodes a 321 amino-acid protein with three tandem related domains of about 100 amino acids. Each domain contains two hydrophobic stretches, which are thought to span the membrane as α-helices. Distant relationship analysis indicates that the protein is highly conserved between species from *Caenorhabditis elegans* to human. HuBMSC-MCP gene is mapped to chromosome 11p11. HuBMSC-MCP mRNA expression is detectable in various human tissues and cell lines. By confocal imaging, HuBMSC-MCP is localized to mitochondria and also detected in the pseudopodial protrusion of human breast adenocarcinoma MCF-7 cells. When transfected into dendritic cells (DC), HuBMSC-MCP could enhance DCs endocytotic capacity. Thus, HuBMSC-MCP is a phylogenetically conserved and widely expressed mitochondrial carrier protein which perhaps associates with mitochondrial oxidative phosphorylation.

Keywords: Mitochondrial carrier protein; Gene cloning; Bone marrow stromal cell; Pseudopodial protrusion; Endocytosis; Dendritic cells

Mitochondria are distinct organelles that not only serve as cells' power sources but also play a key role in the regulation of programmed cell death via release of pro-apoptotic agents and/or disruption of cellular energy metabolism [1,2].

The inner membrane of mitochondria harbors a number of specific carrier proteins responsible for the transport of various metabolites, nucleotides, and cofactors into and out of the matrix space and the induction of apoptosis [3,4]. These related proteins have similar structures and functions and therefore are assigned to a superfamily, the mitochondria carrier superfamily. The members of the superfamily have some common features: (1) the polypeptide chains consist of three tandem related domains of about 100 amino acids; (2) a hydrophobic profile indicates that each domain is probably folded into a common secondary structure consisting of two transmembrane α-helices, linked by an extensive hydrophilic region; and (3) they have a similar molecular mass of approximately 30 kDa [5–10]. Known members of the superfamily include the ADP/ATP carrier [8], the phosphate carrier [11], the mitochondrial folate transporter [12], the calcium-binding mitochondrial carrier protein Aralarl [13], and the mitochondrial oxoglutarate/malate carrier protein [14].

In this study, we identified a novel member of the mitochondria carrier superfamily from human bone marrow stromal cells (BMSC) and designated it as HuBMSC-MCP (Human Bone Marrow Stromal Cell-derived Mitochondrial Carrier Protein). HuBMSC-MCP possesses all of the characteristic sequence features of the superfamily described above and shares homology with human mitochondria folate carrier protein [12] and the calcium-binding mitochondrial carrier protein Aralar [13]. Interestingly, HuBMSC-MCP can enhance endocytosis of dendritic cells (DC).

* Corresponding author. Fax: +86-571-8721-7329.
E-mail address: caoxt@public3.sta.net.cn (X. Cao).

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Materials and methods

Isolation of HuBMSC-MCP cDNA. The full-length cDNA of HuBMSC-MCP was directly isolated from a cDNA library of human BMSC by random sequencing [15]. Briefly, BMSC from patient's bone marrow aspirates were depleted of red blood cells with ammonium chloride. Then the cells were cultured in six-well culture plates (1×10^6 cells/well) in RPMI 1640 supplemented with 10% heat-inactivated FCS, 10% horse serum, 50 μM of 2-ME, 10 mM Hepes (pH 7.4), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. After 24 h, suspending cells were removed and an adherent stromal cell layer was established. On day 14, the stromal cells were stimulated with PMA (100 ng/ml) and LPS (10 μg/ml) for 8 h and then collected for cDNA library construction as described previously [16]. A plasmid cDNA library of pCMV · SPORT6 vector was constructed using the Superscript plasmid system for cDNA synthesis. Plasmid cloning was performed according to manufacturer's instructions (Invitrogen). The full-length cDNA of clone HNE6E12 was found to potentially encode a protein with three conserved mitochondria carrier protein motifs and share homology with the known members of the mitochondrial carrier superfamily, hence the novel protein was designated as HuBMSC-MCP. The full-length sequence is available in the GenBank database with Accession No. AF495714.

Cell culture. The following cell lines were obtained from the ATCC: U-937 (human histocytic lymphoma), K-562 (chronic myelogenous leukemia), NB4 (promyelocytic leukemia), Jurkat (acute T cell leukemia), Reh (acute lymphocytic leukemia), MOLT-4 (acute lymphoblastic leukemia), Raji (Burkitt's lymphoma), PC-3 (prostate adenocarcinoma), MCF-7 (breast adenocarcinoma), HeLa (cervix epithelioid carcinoma), CaoV-3 (ovary adenocarcinoma), and LoVo (colon adenocarcinoma). Standard procedures were used for the cell cultures.

Human peripheral monocyte-derived DC were generated by culturing in GM-CSF and IL-4 as described previously by us [17]. On day 5, DC were harvested and used for mRNA electroporation [18–20]. To produce activated DC, 7 day DC were treated with LPS (100 ng/ml) for 24 h. Human BMSC were cultured in vitro and harvested on day 14. Activated BMSC were prepared in the presence of LPS (10 μg/ml) for 8 h and collected for RT-PCR analysis.

Apoptosis induction. For induction of apoptosis, LoVo and U-937 cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FCS in a humidified 5% CO_2 atmosphere at 37 °C. Twenty-four hours after subculturing, cells were treated with anoxia, 2 mM sodium nitroprusside (SNP), and 500 μM H_2O_2 or 20 mM glucose and harvested at 30 min, 1, 4, 8, 12, and 24 h, respectively, for detection of apoptosis by flow cytometry after staining with FITC-conjugated annexin V and propidium iodide (PI) using a commercially available kit (Annexin V-FITC, BD Biosciences) and expression of HuBMSC-MCP mRNA by RT-PCR. Cells were considered apoptotic when they were annexin V-positive and PI-negative. Hypoxia was induced with the use of a cell culture incubator perfused with 1% O_2/5% CO_2/94% N_2.

RT-PCR and Northern blot. Total cellular RNA was isolated using Trizol reagent (Invitrogen) according to manufacturer's instructions and first strand cDNA was prepared with AMV reverse transcriptase (Promega) using an Oligo$(dT)_{12-18}$ primer. Synthesis of cDNA was checked by RT-PCR using β-actin primers. RT-PCRs with the primers 5′-GAG ACC AAC ATC CGT GAC-3′ (forward) and 5′-CCT CGT CCT TAT GAC TTC-3′ (backward) specific for HuBMSC-MCP cDNA were subjected to denaturing (94 °C, 30 s), annealing (56 °C, 30 s), and extension (72 °C, 30 s) for 30 cycles using a Perkin–Elmer GeneAmp PCR System 9600.

Northern blot filters containing human poly$(A)^+$ RNA from various tissues were purchased from Clontech. The *Kpn*I/*Bam*HI fragment of HuBMSC-MCP cDNA was used as template for probe synthesis. The filters were hybridized with the ^{32}P-labeled HuBMSC-MCP cDNA probe in ExpressHyb hybridization solution (Clontech) according to manufacturer's instructions. After hybridization, the filters were stringently washed at 50 °C for 20 min in 0.1× SSC and 0.1% SDS, and followed by autoradiography. The filters were reprobed with a human β-actin cDNA probe.

Eukaryotic expression of recombinant HuBMSC-MCP protein. To express HuBMSC-MCP fusion protein in eukaryotic cells, the ORF of HuBMSC-MCP was amplified by PCR using forward (5′-CGG GAT CCC ATG GCG ACG GGC GGC CAG C-3′) and reverse (5′-GCG GTA CCG ACT GAG TAC GGT CTT CTA A-3′) primers from BMSC cDNA as a template. The fragment was fused with GFP code region and inserted into vector pcDNA3.1/Myc-His (−) A (Invitrogen). The DNA sequence was confirmed by sequencing. MCF-7 cells were transfected with HuBMSC-MCP expression vector or its mock control vector with LipofectAMINE reagent (Invitrogen) according to manufacturer's instructions. After 3 weeks of resistant screening in the presence of 800 μg/ml G418 (Calbiochem), the stable positive clones were obtained and used for Western blot and confocal analyses.

Western blot. Totally harvested cells were lysed in cell lysis buffer (1% NP-40, 50 mM Tris–HCl, pH 7.8, and 150 mM NaCl) containing PMSF (100 μg/ml), aprotinin (1 μg/ml), and leupeptins (1 μg/ml). The lysates were fractionated by 12% SDS–PAGE gel and transferred onto nitrocellulose membrane. After blocking with 5% nonfat dried milk for 2 h, the membrane was washed three times for 5 min each with TBST (100 mM Tris–HCl, pH 7.6, 0.9% NaCl, and 0.1% Tween 20). Then the membrane was incubated in rabbit anti-GFP polyclonal antibody (Santa Cruz Biotechnology, 1:1000 dilution) overnight at 4 °C. After washing three times for 10 min each with TBST, the membrane was incubated in HRP-coupled anti-rabbit secondary antibody (New England Biolabs, 1:2000 dilution) for 1 h. After washing with TBST as above, the membrane was incubated in LumiGLo (Cell Signaling) for 1 min and then exposed to X-ray film.

Confocal fluorescence microscopy. Cells expressing HuBMSC-MCP–GFP fusion protein were plated and grown on glass coverslips in a six-well plate. For mitochondria staining, living cells were incubated with 200 nM MitoTracker Red CMXRos (Molecular Probes) for 15 min at 37 °C and rinsed three times with prewarmed PBS. MitoTracker-loaded cells were fixed in 4% paraformaldehyde in PBS (room temperature, 15 min), washed, and then used for confocal analysis (Carl Zeiss).

Production of in vitro-transcribed mRNA. The pCIpA102 and pCIpA102/EGFP were kindly provided by Dr. S. Saeboe-Larssen (University of Oslo, Oslo, Norway) [21]. Plasmid for transcribing HuBMSC-MCP was generated by cloning its cDNA into pCIpA102. The plasmids were linearized with *Mfe*I (TaKaRa), purified, and used as DNA templates for the in vitro transcription reaction. Transcription was carried out in a final 20 μl reaction mix at 37 °C for 4 h using the T7 Cap-Scribe Kit (Roche Molecular Biochemicals) to generate 5′m7GpppG-capped in vitro-transcribed mRNA. Purification of mRNA was conducted by DNase I digestion followed by NH_4AC precipitation, according to manufacturer's instructions. The mRNA quality and concentration were assessed by agarose–formaldehyde gel electrophoresis and spectrophotometry [21–23].

mRNA transfection of dendritic cells. Electroporation of mRNA into DC was conducted as described previously with minor modifications [20–22]. Briefly, before electroporation, DC cultured for 5 days were harvested and washed twice with serum-free RPMI 1640. DC were resuspended in serum-free RPMI 1640 to a final concentration of 1×10^7 cells/ml. Subsequently, 300 μl of the cell suspension was mixed with 20 μg HuBMSC-MCP- or EGFP-specific mRNA and electroporated in a 0.2-cm cuvette at 505 V using a BTX ECM-830 square wave electroporator (Genetronics). Two pulses were applied at an interval of 10 s, with each pulse being 99 μs in duration. After electroporation, DC were resuspended in fresh complete medium and incubated at 37 °C. DC transfected to express EGFP were analyzed with a FACSCalibur flow cytometer.

Assay for dendritic cell endocytosis. Twenty hours after electroporation, DC (5×10^5) were incubated with 0.1 mg/ml FITC-dextran (Sigma) either at 4 °C or at 37 °C for 60 min. Then DC were washed twice with ice-cold PBS containing 0.1% sodium azide and 1% BSA and analyzed using a FACSCalibur flow cytometer [24–27].

Results

Sequence analysis of HuBMSC-MCP cDNA and its predicted protein

HuBMSC-MCP cDNA isolated from human BMSC cDNA library was composed of 1433 bp. The complete open reading frame (ORF) of 966 bp potentially encoded a 321-residue protein with a molecular mass of 35.37518 kDa and an isoelectric point of 9.65. Sequence analysis indicated that the protein contained three well-conserved mitochondria carrier protein domains. Each contained about 100 amino acids in length (Fig. 1A). This kind of tripartite structure has been shown to be characteristic of all mitochondrial carrier proteins sequenced to date. In addition, a motif identified as the mitochondrial energy signature (ES) commonly characteristic of mitochondrial carriers was also found in HuBMSC-MCP. The hydrophobic profile of HuBMSC-MCP showed six potential membrane-spanning helices, with both amino and carboxyl termini of the protein and the loops between transmembrane (TM) regions II–III and IV–V possibly facing the cytosol which was consistent with the known mitochondrial carrier proteins (Fig. 1B). A BLAST search with the amino acid sequence revealed significant similarity with proteins of the mitochondrial carrier superfamily. HuBMSC-MCP shared 28% identity and 44% similarity with the carboxyl-terminal half of human calcium-binding mitochondrial carrier protein Aralar, 28% identity and 43% similarity with the full length of human mitochondrial folate transporters (Fig. 2). These results clearly indicated that HuBMSC-MCP is a novel member of the mitochondria carrier superfamily. Genomes map viewer showed that HuBMSC-MCP gene was mapped to chromosome 11p11.

In addition, HuBMSC-MCP protein revealed significant similarities with some proteins from distantly related organisms. For example, it shared 53% identity and 68% similarity with an unknown protein of *Anopheles gambiae* str.PEST (data not shown). Distant relationship was also found with mitochondrial carrier proteins of *Caenorhabditis elegans* [28] and mouse-ear cress *Arabidopsis thaliana*. Therefore HuBMSC-MCP protein represents a highly conserved product among eukaryotic organisms.

Expression pattern of HuBMSC-MCP mRNA

By RT-PCR, we demonstrated HuBMSC-MCP mRNA was expressed in a large number of tumor cell lines including U-937, K-562, NB4, Jurkat, Reh, MOLT-4, Raji, PC-3, MCF-7, HeLa, and CaoV-3 cells, but was not expressed in LoVo cells (Fig. 3A). In human DC and BMSC, HuBMSC-MCP mRNA expression was detected. But HuBMSC-MCP mRNA expression remained unchanged in the activated DC and BMSC after LPS stimulation (Fig. 3B).

The mRNA expression pattern of HuBMSC-MCP in human tissues was examined by Northern blot analysis (Fig. 4). The abundant expression of HuBMSC-MCP was observed in testis and skeletal muscle, and moderate expression observed in heart, brain, liver, kidney, prostate, colon (mucosal lining), and peripheral blood

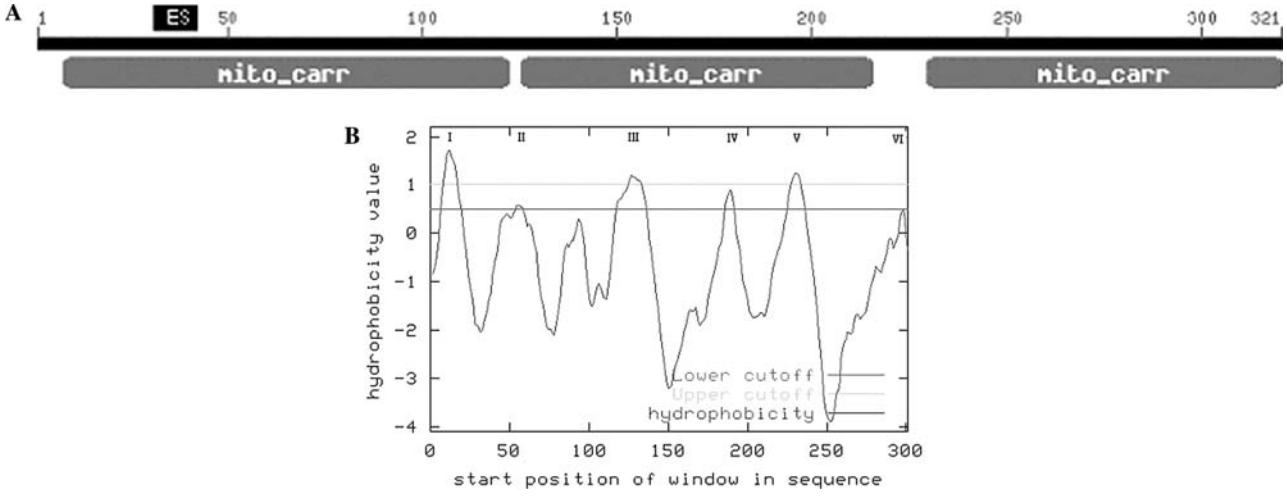

Fig. 1. Structure of HuBMSC-MCP protein. (A) Schematic drawing of HuBMSC-MCP protein. The location of the mitochondrial carrier protein motifs (mito-carr) and the mitochondrial energy signature motif (ES) are showed. (B) Hydropathy plot of HuBMSC-MCP protein. Predicted TM domains are indicated in roman numerals. The codon number (abscissa) is plotted against the hydrophobicity (positive values) or hydrophilicity (negative values) of a sliding window of residues using the TopPred 2 algorithm.

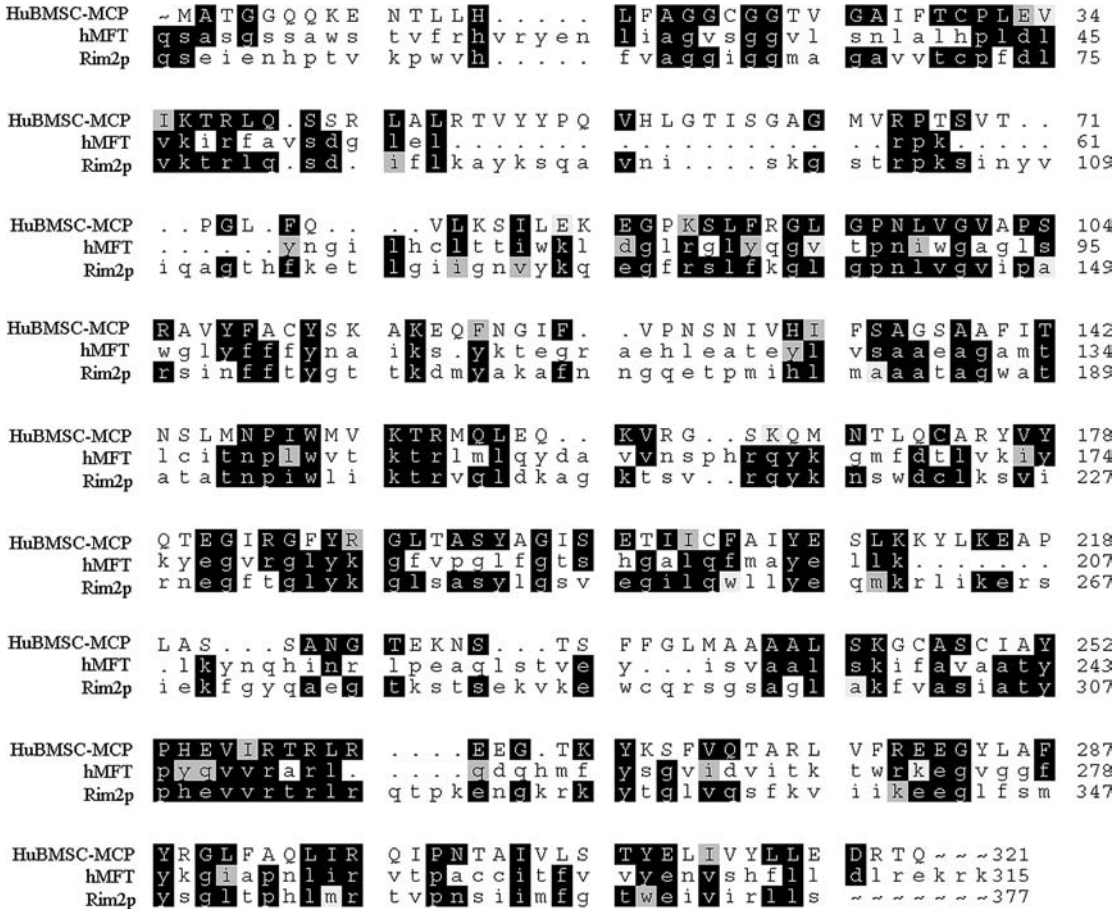

Fig. 2. Multiple alignment of HuBMSC-MCP with the human mitochondrial folate transporter/carrier (hMFT), and uncharacterized *Saccharomyces cerevisiae* mitochondrial carrier protein (Rim2p). Identities (■) and positives (▒) are shown. The alignment was performed with the GCG package.

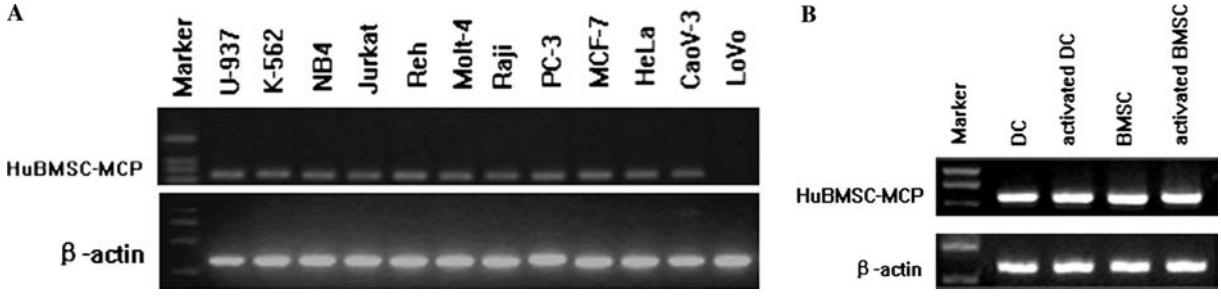

Fig. 3. RT-PCR analysis of HuBMSC-MCP mRNA expression. (A) Various tumor cell lines were subjected to RT-PCR analysis of HuBMSC-MCP expression. (B) Freshly isolated human DC, BMSC, and LPS activated DC and BMSC were subjected to RT-PCR analysis of HuBMSC-MCP expression. Human β-actin was amplified as positive control.

leukocyte (PBL), but no expression in placenta, lung, pancreas, spleen, thymus, ovary or small intestine.

No relationship of HuBMSC-MCP mRNA expression with apoptosis

In recent years, many studies have demonstrated that mitochondria play an essential role in the process of apoptosis [29–32]. Mitochondrial depolarization marks an irreversible commitment of the cell to apoptosis [33]. Since HuBMSC-MCP was one of the mitochondrial inner membrane proteins, we investigated whether it may be related to apoptosis. We selected two cell lines: LoVo and U-937. In normal condition, U-937 expressed while LoVo did not express HuBMSC-MCP. Both cell lines were treated with several pro-apoptotic stimuli and the changes of HuBMSC-MCP expression during apoptosis were observed. Apoptosis was detected in LoVo

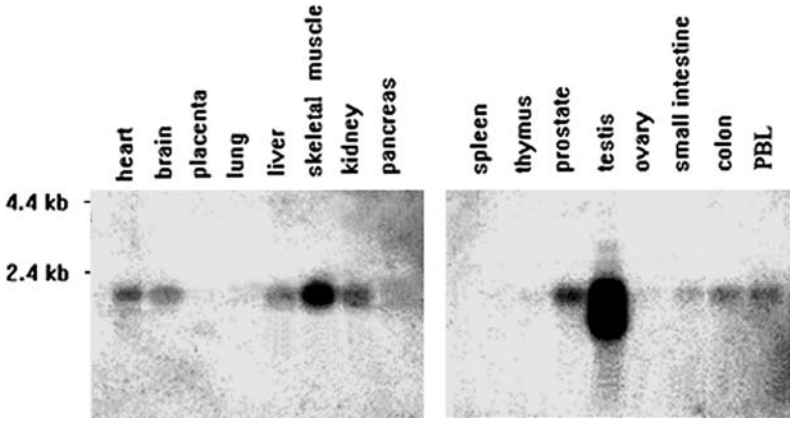

Fig. 4. Northern blot analysis of HuBMSC-MCP expression in normal tissues. Human multiple tissue Northern blots (Clontech) were probed as described under "Materials and methods." The tissue sources of mRNA are indicated above each lane. Molecular size standards are indicated in kilobases.

and U-937 cells were with annexin V positive and PI negative (data not shown), while no changes of HuBMSC-MCP mRNA expression were observed, as analyzed by RT-PCR. These suggested that HuBMSC-MCP might, in our present experimental system, have no direct relationship with apoptosis induced by those stimuli mentioned above.

Expression of HuBMSC-MCP protein in HuBMSC-MCP-transfected cells

The expression of HuBMSC-MCP–GFP fusion protein was detected in MCF-7 cells transfected with HuBMSC-MCP–GFP expression vector. A ~64 kDa protein was detected in stable clone of transfected MCF-7 cells by Western blotting with anti-GFP antibodies (Fig. 5). The molecular mass of GFP protein is about 27 kDa, so HuBMSC-MCP protein was about 34 kDa that is in reasonable agreement with the value of 35.375 kDa predicted by ProtParam tool.

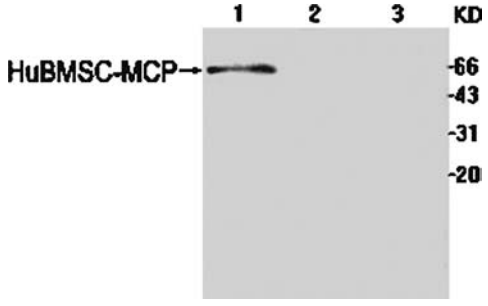

Fig. 5. Western blot analysis of HuBMSC-MCP protein expression. HuBMSC-MCP cDNA transfected MCF-7 cells were subjected for Western blot analysis of HuBMSC-MCP protein expression (lane 1). MCF-7 cells transfected pcDNA3.1 (lane 2) and parental MCF-7 cells were subjected to negative controls (lane 3). Protein markers are shown on the right.

Intracellular distribution of HuBMSC-MCP in transfected MCF-7 cells

The intracellular distribution of HuBMSC-MCP protein was analyzed in the stable clone of HuBMSC-MCP–GFP transfected MCF-7 cells. Results showed that HuBMSC-MCP–GFP was localized to numerous small granular structures throughout the cytoplasm (Fig. 6A). This distribution was similar to the pattern of mitochondria labeled by mitochondrial-specific fluorescent dye MitoTracker Red CMXRos. Examination of the merged confocal images revealed a high degree of coincidence between HuBMSC-MCP–GFP and mitochondria distribution. The data demonstrated that HuBMSC-MCP protein is targeted to mitochondria. It should be noted that HuBMSC-MCP overexpression induced changes of the mitochondrial morphology from a filamentous to a punctate pattern, as observed also for cells overexpressing mitochondrially located Bax protein [34].

Pseudopodial protrusion is a prominent feature of actively motile cells in vitro and invading tumor cells in vivo [35–37]. In the pseudopodial protrusion of MCF-7 cells, HuBMSC-MCP–GFP was detected with co-localized to mitochondria (Fig. 6B).

Electroporation of DC with in vitro transcribed mRNA is a highly efficient transfection method

EGFP mRNA was used as a tool to test the transfection efficiency and the level and kinetics of the reporter gene expression. The expression level reached a maximum 20 h after electroporation and decreased slowly (data not shown). Fig. 7 showed a representative flow cytometric analysis of EGFP expression by DC 20 h after electroporation. Twenty hours after transfection, 71% of DC expressed EGFP with an MFI of 80%.

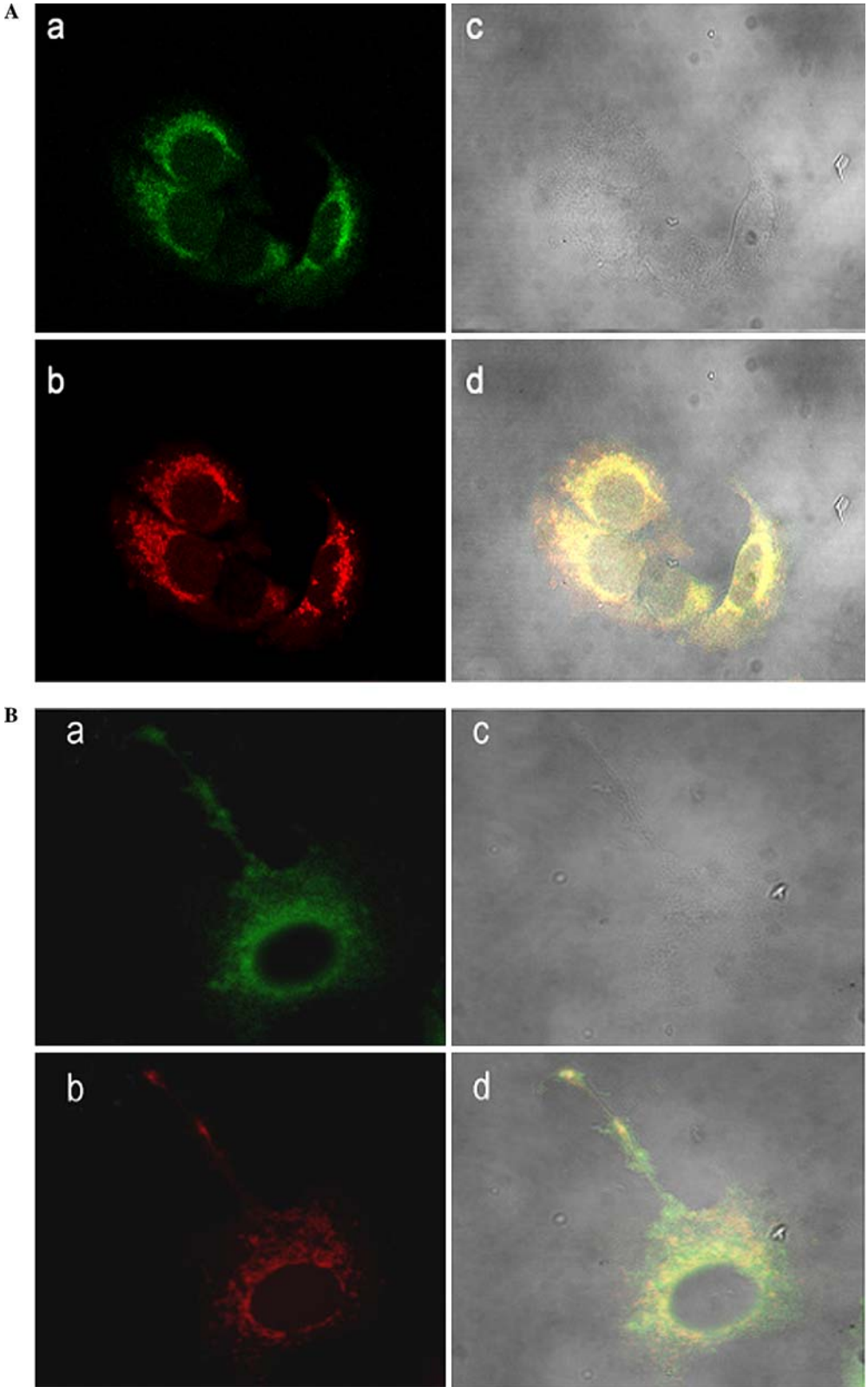

Fig. 6. Localization of HuBMSC-MCP protein in transfected MCF-7 cells. (A) Localization HuBMSC-MCP-GFP to mitochondria. (a) HuBMSC-MCP-GFP is localized to numerous small granular structures throughout the cytoplasm (green). (b) Mitochondria stained with MitoTracker (red). (d) An overlap of the green and red images. Sites of co-localization appear yellow. (c) HuBMSC-MCP-GFP transfected MCF-7 cells. (B) Localization of HuBMSC-MCP-GFP in the pseudopodial protrusion of MCF-7 cells. (a) HuBMSC-MCP was detected in the pseudopodial protrusion of MCF-7 cells (green). (b) Mitochondria stained with MitoTracker (red). (d) HuBMSC-MCP-GFP was co-localized to mitochondria. (c) HuBMSC-MCP-GFP transfected MCF-7 cells.

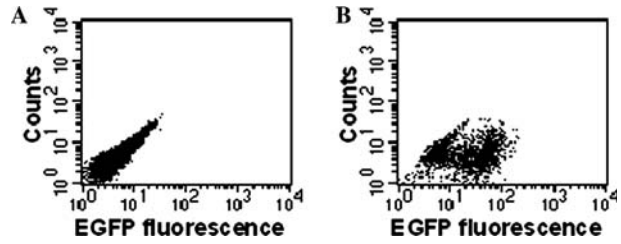

Fig. 7. Transfection efficiency of mRNA-electroporated dendritic cells. (A) Mock electroporation. (B) EGFP mRNA electroporation. Twenty hours after electroporation of day 5 DC with 20 μg EGFP mRNA, a transfection efficiency of 71% was obtained with a mean fluorescence intensity of 80%. Similar results were obtained in several (>3) separate experiments.

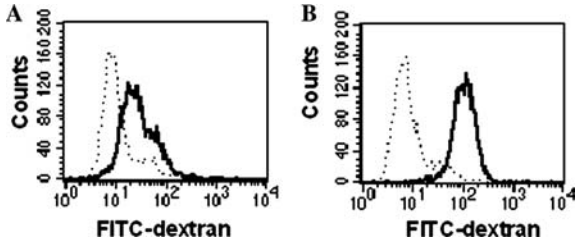

Fig. 8. Endocytosis of FITC-dextran by DC transfected with HuBMSC-MCP mRNA. Twenty hours after electroporation, DC were incubated with FITC-dextran for 60 min at 4 °C (dotted lines) or 37 °C (bold lines). (A) Endocytosis of FITC-dextran by DC mocked electroporation. (B) Endocytosis of FITC-dextran by DC electroporated with HuBMSC-MCP mRNA.

HuBMSC-MCP enhances the endocytosis of dendritic cells

DC are professional antigen-capturing and -presenting cells that play important roles in initiation and modulation of immune responses. Immature DC have unsurpassed capacity to take up exogenous antigens. After endocytosis, the antigens are processed and presented on the surface of DC which lead to the activation of Ag-specific T cells. To investigate the impact of HuBMSC-MCP on DC endocytosis, HuBMSC-MCP mRNA was transfected into DC and the endocytosis of DC was detected by the uptake of FITC-dextran. As shown in Fig. 8, 20 h after transfected with HuBMSC-MCP mRNA, the endocytic capacity of DC was significantly increased.

Discussion

The inner membrane of mitochondria contains several different carrier proteins involved in the transport of anions and protons across the membrane, which transport cations (glutamine, carnitine, and ornithine), anions (ATP/ADP, Pi, oxoglutarate, and citrate), and flavins as well as the H^+ ions involved in the uncoupling reaction. These proteins with common structural and functional features constitute a superfamily-mitochondrial carrier superfamily [5–10]. In this study, we isolated a novel member of this superfamily, HuBMSC-MCP that encodes a protein of 321 amino acids with many common properties of the superfamily. The protein has a predicted molecular mass of 35.378 kDa which is in reasonable agreement with the value of about 34 kDa estimated by Western blot and has predicted three repeated domains about 100 amino acids long in its primary structure. Each domain consists of two TM domains (I–VI). The regions between TM domains I and II, III and IV, and V and VI are relatively hydrophilic and were proposed to form extensive extra-membrane domains. In other members of the superfamily, a mitochondrial ES (energy signature) motif, PX(D/E)X(L/I/V/A/T)(R/K)X(L/R/H)(L/I/V/M/F/Y)(Q/G/A/I/V/M), is found immediately after TM domains I, III, and V. The motif is thought to be important in targeting carriers by way of the Tim10/Tim12/Tim22 pathway to the mitochondrial inner membrane [38,39]. In HuBMSC-MCP protein, there is only one ES motif following TM domain I. Steven et al. reported that the first ES motif was more conserved, while the second and the third motifs had some deviations [12]. This perhaps shows that the first ES motif is more important for mitochondrial targeting, translocation across the outer mitochondrial membrane, and insertion into the inner membrane.

By homology search, we have found a striking relationship of HuBMSC-MCP protein with many lower species such as *C. elegans*, *A. thaliana*, and *An. gambiae* str.PEST. This suggested that HuBMSC-MCP is phylogenetically conserved and may be important for cell function.

Using confocal imaging, we not only localized HuBMSC-MCP to mitochondria but also observed an interesting phenomenon that HuBMSC-MCP was also detected in the pseudopodial protrusion of MCF-7 cells. Pseudopod protrusion is a characteristic of migrating tumor cells as they traverse vascular subendothelial basement membranes to establish metastasis [40]. The formation and extension of pseudopod protrusion is regulated by a wide variety of agents including growth factors, extracellular matrix components, and motility factors, some of which are secreted by tumor cells, such as tumor autocrine motility factor (AMF) [41–44]. Recently, chemokine receptors CXCR4 and CCR7 are also reported to be able to mediate the pseudopodia formation of breast cancer cells. CXCR4 and CCR7 are highly expressed in human breast cancer cells (including MCF-7) [45]. Their respective ligands CXCL12/SDF-1α and CCL21/6Ckine are highly expressed in organs such as the regional lymph nodes, bone marrow, lung, and liver—the first destinations of breast cancer metastasis. In breast cancer cells, signaling through CXCR4 or CCR7 mediates actin polymerization/cross-linking and

osmotic swelling at the site of a chemotactic stimulus. These two forces combine to drive the formation and extension of pseudopod protrusion. And this subsequently induces invasion and metastasis of breast cancer cells. Signal transduction and the formation of pseudopod protrusion all need energy, while HuBMSC-MCP was detected in the pseudopod protrusion of MCF-7 cells and co-localization to mitochondria, so HuBMSC-MCP might transport one kind of substrate related to mitochondrial oxidative phosphorylation and provide energy for tumor cell metastasis.

DC are the most potent antigen presenting cells and are capable of initiating immune responses in vitro and in vivo. Efficient endocytosis is important for DC to fulfill their sentinel function in immunity [18]. Using the most efficient transfection method—mRNA electroporation, we introduced HuBMSC-MCP into DC and found that expression of HuBMSC-MCP could enhance the endocytotic capacity of DC. Since endocytosis needs energy, we postulated that HuBMSC-MCP might associate with mitochondrial oxidative phosphorylation and provide energy for DC endocytosis. The relationship between HuBMSC-MCP and the endocytotic capacity of DC is under further investigation.

Although no relationship of HuBMSC-MCP and apoptosis was shown in our experimental system, the functions of HuBMSC-MCP need further investigation.

In summary, we cloned a novel mitochondrial carrier protein HuBMSC-MCP that has all the features of mitochondrial carrier superfamily and shares homology with the known mitochondrial carrier proteins. HuBMSC-MCP is localized to mitochondria and also detected in the pseudopodial protrusion of MCF-7, breast adenocarcinoma cells. When over-expressed in DC, HuBMSC-MCP enhances endocytic capacity of DC. These data suggest that HuBMSC-MCP belongs to mitochondrial carrier superfamily and might transport substrate related to oxidative phosphorylation. Further biocharacterization of HuBMSC-MCP will be helpful to define its actual role in mitochondrial oxidative phosphorylation.

Acknowledgments

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