MDC9, a Widely Expressed Cellular Disintegrin Containing Cytoplasmic SH3 Ligand Domains

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Abstract. Cellular disintegrins are a family of proteins that are related to snake venom integrin ligands and metalloproteases. We have cloned and sequenced the mouse and human homologue of a widely expressed cellular disintegrin, which we have termed MDC9 (for metalloprotease/disintegrin/cysteine-rich protein 9). The deduced mouse and human protein sequences are 82% identical. MDC9 contains several distinct protein domains: a signal sequence is followed by a prodomain and a domain with sequence similarity to snake venom metalloproteases, a disintegrin domain, a cysteine-rich region, an EGF repeat, a membrane anchor, and a cytoplasmic tail. The cytoplasmic tail of MDC9 has two proline-rich sequences which can bind the SH3 domain of Src, and may therefore function as SH3 ligand domains. Western blot analysis shows that MDC9 is an ~84-kD glycoprotein in all mouse tissues examined, and in NIH 3T3 fibroblast and C2C12 myoblast mouse cell lines. MDC9 can be both cell surface biotinylated and 125I-labeled in NIH 3T3 mouse fibroblasts, indicating that the protein is present on the plasma membrane. Expression of MDC9 in COS-7 cells yields an 84-kD protein, and immunofluorescence analysis of COS-7 cells expressing MDC9 shows a staining pattern that is consistent with a plasma membrane localization. The apparent molecular mass of 84 kD suggests that MDC9 contains a membrane-anchored metalloprotease and disintegrin domain. We propose that MDC9 might function as a membrane-anchored integrin ligand or metalloprotease, or that MDC9 may combine both activities in one protein.
also integrin ligands, they may play an important role in some of these processes.

Here we report the cloning and cDNA sequencing, and the initial biochemical characterization of a widely expressed cellular disintegrin referred to as metalloprotease/disintegrin/cysteine-rich protein 9 (MDC9). MDC9 is highly conserved between mouse and human, containing all protein domains that are found in other cellular disintegrins, and also includes a proline-rich cytoplasmic tail that may function as an Src homology 3 ligand domain. The apparent molecular mass of 84 kD in all tissues and cells examined suggests that mouse MDC9 contains a membrane-anchored metalloprotease and disintegrin domain. These results are discussed in the context of the predicted role of MDC9 in cell–cell interactions.

Materials and Methods

Animals and Reagents

Reagents, including Taq polymerase, were obtained from Boehringer Mannheim (Indianapolis, IN), unless indicated otherwise. Mice (nontransgenic animals) were obtained from the transgenic breeding facility of the Sloan Kettering Institute (New York, NY). Rabbits were purchased from Hazeltown (Denver, PA). Radiolabeled nucleotides were supplied by Amersham Corp. (Arlington Heights, IL).

cDNA Cloning

A sequence tag encoding the partial disintegrin domain of MDC9 (sequence tag No. 16-2 (41)) was used to screen a mouse lung cDNA library (Stratagene, La Jolla, CA) under high stringency conditions as described (8), which yielded three identical clones with inserts of 2,330 bp. One cDNA clone was sequenced completely (Sequenase, USB, Cleveland, OH) in both orientations by a primer walk. Since the cDNA insert size of 2.33 kb did not match that observed for the mRNA (~4 kb), we constructed a new mouse lung cDNA library (Stratagene ZAP cDNA library synthesis kit, according to the manufacturer's instructions, see also reference 24). The unamplified cDNA library was screened under high stringency conditions with the partial 2.33 kb MDC9 clone, and yielded more than 100 positive plaques in the primary screen. PCR analysis of 80 positive plaques with an antisense primer that annealed close to the 5' end of the 2.33 kb clone, and the vector specific T3 primer, revealed that the cDNA of 10 clones extended between 800 and 850 bp past the 5' end of the shorter 2.33 kb clone. One cDNA clone was purified, subjected to in vitro excision, and sequenced as described above. This clone contained a cDNA insert of 3,851 bp, encoding for an open reading frame of 845 amino acid residues (GenBank accession No. U41765).

To clone the full-length cDNA for human MDC9, we synthesized a human cDNA library from MDA-MB-468 mammary epithelial carcinoma cells (ATCC HTB-132) as described above. The MDA-MB-468 cDNA library was screened with the full-length cDNA of mouse MDC9, and more than 50 positive plaques were identified. Clones with the longest 5' extension were identified by PCR. (see above), using as antisense primer the most 5' sequence of a partial human MDC9 sequence (GenBank accession No. D14665). One clone with a cDNA insert of 3,866 bp was sequenced on both strands, and contained an open reading frame of 845 amino acid residues (GenBank accession No. U41766). cDNA- and translated-protein sequences were analyzed using MacVector software (Kodak IBI, New Haven, CT). Sequence alignments and comparisons among cellular disintegrin proteins were performed using DNA STAR MEGALIGN software.

Northern Blot

Total RNA was isolated from mouse lung, heart, liver, lung, skeletal muscle, brain, spleen, kidney and testis, and from C2C12 mouse muscle cells as described (9). For each tissue, 15 μg of RNA were run per lane, and the integrity of the ribosomal RNA of each sample was monitored on a separate gel. Human tissue Northern blots were obtained from Clontech (Palo Alto, CA). Northern blot analysis was performed with the appropriate 32P-labeled mouse or human MDC9 cDNA probe under high stringency as previously described (41).

Blot Overlay with Biotinylated SH3 Domains of Src and Abl

Three separate gtt-fusion proteins containing different portions of the mouse MDC9 cytoplasmic tail were constructed by PCR using gene-specific primers with added restriction sites to allow direct fusion with gtt. Construct P1 encoded for amino acid residues 808 to 824 and included the potential SH3 ligand domain RPPPPQPO, and construct P2 contained amino acid residues 923 to 945, including the second potential SH3 ligand domain RPAPAPP. In construct P1+2, both SH3 ligand domains were combined by a fusion with amino acid residues 908 to 945. The cDNA for each construct was sequenced to rule out any mutations possibly introduced by PCR. All three fusion proteins, and gtt alone, were expressed in BL26 cells (Novagen, Madison, WI). To isolate the fusion proteins, bacteria were disrupted in a French press homogenizer, and gtt-fusion proteins were allowed to bind to a glutathione matrix (Pharmacia LKB Biotechnology, Piscataway, NJ), and eluted with sample loading buffer. As probes for the SH3 ligand domain, gtt-fusion proteins with either the SH3 domain of Src or Abl (kindly supplied by Dr. Margaret Chao), were isolated as described above, and biotinylated with NHS-LC-biotin (Pierce, Rockford, IL) following the manufacturer’s instructions. A blot of the biotinylated Src- and Abl-SH3 fusion proteins was probed with HRPlabeled streptavidin (Pierce), and the level of biotinylation was found to be comparable between the two proteins (not shown). For the actual blot overlay experiment, MDC9 gtt-fusion proteins were run on a 12% polyacrylamide gel and transferred to nitrocellulose. After blocking with 5% dry milk in water, the blot was incubated with the biotinylated Src- or Abl-SH3 fusion proteins (0.1 μg/ml), followed by incubation with HRPlabeled streptavidin. Bound HRPlabeled streptavidin was detected by chemiluminescence (ECL, Amersham) and exposure of Kodak XAR autoradiography film (New Haven, CT).

Antibodies

Polyclonal antisera were raised against a gtt-fusion protein with the 114 COOH-terminal amino acid residues of the cytoplasmic tail of mouse MDC9 (constructed by PCR as described above for shorter gtt-cytotail fusion proteins). Ribi adjuvant (RIBI Immunochemical Research Inc.) was used for immunizations according to established protocols (15). To demonstrate that the MDC9-cyto IgG specifically recognize MDC9, we attempted to affinity purify antibodies using the gtt-fusion protein coupled to CNBr-activated Sepharose beads (Pharmacia Biotech). Although several different elution conditions were tested, we were unable to recover sufficient amounts of affinity purified IgG. As an alternative control for the specificity of the gtt-cytotail antibodies, we immunodepleted protein A purified gtt-cyto IgG of antibodies reacting with gtt alone, or of antibodies reacting with the gtt-cyto fusion protein, using gtt or gtt-cytotail fusion protein coupled to CNBr-activated CL4B beads. Immunodepletion of IgG reactive with gtt or with the gtt-cyto fusion protein was confirmed by probing Western blots of gtt or gtt-cyto fusion proteins.

Western Blot Analysis

Mouse tissues were dissected immediately after euthanasia (performed according to the guidelines of the American Veterinary Society), and homogenized in 10 ml lysis buffer per gram of tissue (1% NP-40, 1 mM EDTA, in PBS and protease inhibitors [7]) using a Polytron homogenizer (Kinematica, Littau, Switzerland). Mouse sperm were isolated from the distal cauda epididymis as described (35), washed in PBS, and lysed in lysis buffer at a concentration of 5 × 107 sperm per ml. NIH 3T3 mouse fibroblasts, and C2C12 mouse myoblasts were lysed at a concentration of 4 × 106 cells per ml. All lysates were spun at 27,000 g for 30 min, and the supernatant was incubated with 1 ml Con A-Sepharose (Pharmacia) per 10 ml extract for 1 h at 4°C. Bound glycoproteins from all tissues were eluted with 1 M α-methyl-D-mannose (Sigma Chem Co., St. Louis, MO) in PBS, 1% NP-40, 5 mM EDTA, and protease inhibitors, for 10 min at 37°C. Sample buffer was added to the eluted glycoproteins, and all samples were heated to 95°C for 5 min, with or without 50 mM DTT, as indicated. Gly-
coproteins from mouse sperm, C2C12 mouse myoblasts, and NIH 3T3 fibroblast were eluted by boiling in sample loading buffer. Proteins were separated by SDS-PAGE (25), and transferred to nitrocellulose (Schleicher and Schuell, Keene, NH) using a semidry blotting apparatus (E and K, Saratoga, CA). Blotted protein samples were treated as described for Western blot analysis (7), except that a horseradish peroxidase–coupled secondary antibody (Promega Biotech, Madison, WI) was used in combination with a chemiluminescence detection system (ECL, Amersham) and Kodak XAR autoradiography film.

Expression of MDC9 in COS-7 Cells

The full-length gene for mouse MDC9 was cloned into a pcDNA3 expression vector (Invitrogen, San Diego, CA). MDC9 cDNA was transfected into COS-7 cells (kindly provided by Dr. M. Jasin) using lipofectin (GIBCO/BRL) to obtain a transiently transfected expression. Culture of MDC9 in COS-7 cells was monitored by Western blot as described above.

Cell Surface Labeling

NIH 3T3 mouse fibroblasts were biotinylated with the nonmembrane-permeable biotinylation reagent NHS-LC-biotin (Pierce) as described in reference 22, except that the cells were not lysed and removed from the dish until after the 45-min biotinylation reaction on ice, and the subsequent quenching of the labeling reaction with 0.1 M glycine. Cell surface iodination was performed by incubating a confluent 10-cm tissue culture dish of NIH 3T3 fibroblasts with 2 ml PBS containing 120 μg lactoperoxidase, 60 μl of 0.03% fresh H2O2, and 1 mCi 125I at 4°C for 10 min. The reaction was terminated by addition of 10 ml PBS, 5 mM KI. Extracts of biotin-labeled or 125I-labeled NIH 3T3 cells were immunoprecipitated with MDC9-specific antibodies, or control antibodies as described in reference 42. Immunoprecipitated 125I-labeled material was run on SDS-PAGE, which was dried and used to expose Kodak XAR film by autoradiography, whereas immunoprecipitated biotinylated material was transferred to nitrocellulose, probed with horseradish peroxidase–labeled streptavidin, and detected using a chemiluminescent detection system as described above.

Immunofluorescence Staining

COS-7 cells transfected with MDC9 were grown on 8-well chamber slides (Nunc), fixed with 4% paraformaldehyde, and permeabilized with 1% NP-40 in PBS. MDC9-cyto antibodies, or gst-cyto–depleted control antibodies were added at a concentration of 1:150 in PBS, 0.2% NP-40, followed by a secondary rhodamine-labeled goat anti-rabbit antibody (Boehringer Mannheim Corp., Indianapolis, IN).

Results

Cloning and Sequencing of Mouse and Human MDC9 cDNA

In a previous study, the polymerase chain reaction was used to demonstrate that several characteristic sequence tags for cellular disintegrin proteins exist in the mouse (41). A cDNA clone of 3.851 bp coding for a full-length open reading frame (including sequence tag 16#2 [41]) was isolated from a mouse lung cDNA library as described in Materials and Methods. The protein encoded by this cDNA was termed MDC9, for metalloprotease disintegrin cysteine-rich protein 9. The deduced protein sequence of mouse MDC9 contains 845 amino acid residues (Fig. 1 A) including a predicted hydrophobic transmembrane domain (Fig. 1 B) and six potential N-linked glycosylation sites. The length of the MDC9 cDNA insert is consistent with the size of MDC9 mRNA, which is close to 4 kb as assayed by Northern blot analysis (Fig. 2). Mouse MDC9 is highly related to a partial human cDNA sequence deposited in GenBank (D14665; HUMORF_09). To compare mouse MDC9 with the full-length human MDC9, we cloned and sequenced human MDC9 from a cDNA library constructed from MDA-MB-468 human mammary epithelial cancer cells. The longest human MDC9 clone had an insert of 3,866 bp with an open reading frame encoding for a protein with 819 amino acid residues. An alignment of the deduced protein sequences of human and mouse MDC9 is shown in Fig. 1 A. The human and mouse MDC9 proteins are 82% identical, and contain all protein domains that are characteristic of cellular disintegrins. A signal sequence is followed by a prodomain, a metalloprotease domain, a disintegrin domain, a cysteine-rich region, an EGF repeat, a transmembrane domain, and a cytoplasmic tail. A comparison of mouse MDC9 with selected known cellular disintegrin proteins revealed an overall sequence similarity of 29% with guinea pig fertilin β, 27.5% with guinea pig fertilin α (8, 44), 20.5% with mouse MS2 (49), and 20.6% with rat EAPI (34) (data not shown, for examples of cellular disintegrin sequence alignments see references 8, 44, 45).

The hydrophobicity plot of mouse MDC9 indicates that, in addition to a hydrophobic transmembrane domain, a short hydrophobic region is present in precisely the same relative position as the predicted fusion peptide of fertilin α (Fig. 1 B). The short hydrophobic segment of both mouse MDC9 (not shown) and fertilin α can be modeled as amphipathic α helix (8). However, unlike guinea pig fertilin α, which is only expressed in testis, both human and mouse MDC9 are expressed in all tissues examined by Northern blot analysis (Fig. 2).

Western Blot and Immunofluorescence Analysis

To analyze the domain organization of MDC9 in mouse tissues and cell lines, we raised antibodies against a gst–fusion protein with the cytoplasmic tail of MDC9. For all experiments involving MDC9-reactive antibodies, we used antibodies raised against the gst-MDC9-cytoplasmic tail, and depleted of IgG that bind to the gst moiety only (referred to as MDC9-cyto IgG, see Materials and Methods). As control IgG, we depleted an identical MDC9-gst-cyto tail IgG sample of all antibodies reacting with the gst-MDC9-cyto fusion protein.

Since mouse MDC9 was cloned from a lung cDNA library, the initial characterization of MDC9 was performed on mouse lung tissue. A Western blot of a mouse lung extract probed with MDC9-cyto IgG (Fig. 3 A, lane 1) showed a strong band of 84 kD, and two weaker bands of 115 and 60 kD. As mouse MDC9 is predicted to have six N-linked glycosylation sites, it was expected to bind to the lectin Con A. In a mouse lung extract depleted of glycoproteins with Con A, the bands of 84 and 115 kD were not visible, whereas the 60-kD band was still detectable (Fig. 3 A, lane 2). Both the 84-kD and 115-kD bands were recovered from Con A by competitive elution with α-methyl-d-mannose (Fig. 3 A, lane 3). Due to the high cysteine content of MDC9 (45 extracellular cysteine residues), reduced and nonreduced MDC9 should show a clearly different mobility on SDS-PAGE. In a sample of mouse lung glycoproteins analyzed under nonreducing conditions, the MDC9-cyto IgG recognized a band of 76 kD, and a weaker 115-kD band (Fig. 3 A, lane 5). Fig. 3 A, lane 4 contains nonreduced mouse lung glycoproteins run adjacent to the reduced sample in lane 3. Therefore, proteins in the left part
Figure 1. Comparison of the amino acid sequence of mouse and human MDC9. (A) Alignment of the mouse and human MDC9 amino acid sequences (produced with DNA STAR MEGALIGN). A potential signal peptidase cleavage site (39) is marked with a large arrowhead. The predicted NH2 termini (44) of individual disintegrin protein domains are indicated by a vertical bar and a small horizontal arrowhead above the aligned sequences. The metalloprotease consensus sequence is indicated by a box with shaded outlines. The position of the predicted integrin-binding sequence TSEC within the disintegrin domain is underlined. Potential N-linked glycosylation sites of mouse MDC9 are marked with an asterisk. Cytoplasmic proline rich sequences that are possibly SH3 ligand domains are boxed and marked P1 and P2. (B) Hydrophobicity plots of mouse MDC9 and of guinea pig fertilin α beginning at the NH2 terminus of the disintegrin domain (produced with DNA strider 1.1 software). A bar marks the position of the putative fusion peptide of fertilin α, and of the hydrophobic sequence in MDC9.
Figure 2. Northern blot analysis of mouse and human MDC9 expression. Northern blots of mRNA isolated from (A) mouse tissues and proliferating C2C12 mouse myoblasts (15 μg total RNA loaded per lane) and (B) human tissues (Clontech human tissue Northern blots 1 and 2) were probed under high stringency conditions with the appropriate 32P-labeled mouse or human cDNA. The mRNA tissue source is indicated above each lane.

of lane 4 are partially reduced, which allows the 76-kD band (Fig. 3 A, lane 5, nonreduced sample) to be traced into the 84-kD band (Fig. 3 A, lane 3, reduced sample). An identical blot probed with control IgG revealed no binding to the 76-kD (nonreduced) or 84-kD (reduced) band, whereas the 115-kD band was still visible (Fig. 3 A, bottom panel).

To analyze the expression and processing of MDC9 in different mouse tissues and cell lines, a Western blot of several Con A–purified mouse tissue extracts run under reducing conditions was probed with MDC9-cyto IgG, or control IgG. In all tissues examined, as well as in mouse sperm, NIH 3T3 fibroblasts and C2 muscle cells, an ~84-kD protein was observed (Fig. 3 B, top panel). The relative tissue specific expression levels of the MDC9 protein (Fig. 3 B, top panel) and mRNA (Fig. 2 A) are different, which could result from a different detergent extractability of MDC9 depending on the tissue source, or could indicate that MDC9 expression is also regulated at the posttranscriptional level. The slight variations in apparent molecular weight might be explained by different carbohydrate modifications in different tissues. Both the NIH 3T3 and C2C12 (47) mouse cell lines also contain a 120-kD band that is recognized by MDC9-cyto IgG, but not by control IgG, and may represent a small amount of MDC9 precursor protein.

As a further confirmation that the 84-kD protein recognized by MDC9-cyto IgG in mouse tissues and cells is indeed MDC9, we transfected MDC9 cDNA into COS-7 cells, and compared extracts of MDC9 expressing COS-7 cells with mouse lung glycoproteins by Western blot (Fig. 3 C, top panel). Whereas extracts of nontransfected (Fig. 3 C, lane 4), or vector-transfected COS-7 cells (Fig. 3 C, lane 3) did not contain an 84-kD band recognized by MDC9-cyto IgG, extracts of MDC9 expressing COS-7 cells (Fig. 3 C, lane 2) contained a protein very similar to the 84-kD glycoprotein in mouse lung (Fig. 3 C, lane 1). A weaker band of 65 kD, which is only visible in MDC9-transfected COS-7 cells, may represent a proteolytically processed form of MDC9. In an identical blot probed with control IgG the 84-kD and 65-kD bands were not visible (Fig. 3 C, bottom panel). The control IgG further demonstrate that the 60-kD band in lane 1, and the 30-kD bands in lanes 2–4 are not specifically recognized by MDC9-cyto IgG. Based on
Figure 3.
the data presented above, we conclude that the 84-kD protein recognized on Western blots of mouse tissues under reducing conditions is MDC9.

Considering that cellular disintegrins are predicted to play a role in cell–cell interactions, MDC9 should be detectable at the cell surface. To confirm that MDC9 is present at the cell surface, NIH 3T3 fibroblasts were cell surface labeled by two different methods, biotinylation with the water soluble reagent NHS-LC-biotin, or 125I iodination catalyzed by lactoperoxidase. Labeled NIH 3T3 cell extracts were immunoprecipitated with MDC9-cyto IgG, or control IgG. Fig. 3 D shows that a biotinylated protein (lane 2), or a 125I-labeled protein (lane 3) of 84 kD can be immunoprecipitated from NIH 3T3 cells with the MDC9-cyto IgG, but not with control IgG (Fig. 3 D, lanes 1 and 4).

Although both labeling methods confirm that MDC9 is indeed expressed on the cell surface of NIH 3T3 fibroblasts, immunofluorescence staining of fixed and permeabilized NIH 3T3 fibroblasts and C2C12 cells with the MDC9-cyto IgG did not reveal a clear cell surface staining. This result may be due to an insufficient amount of MDC9 on the plasma membrane for detection, or to obstruction of cytoplasmic epitopes. When immunofluorescence analysis with MDC9-cyto IgG was performed on COS-7 cells that were transiently transfected with MDC9, a clearly visible staining of transfected cells was observed. As shown in Fig. 4 A, staining extended into the cell boundary and into processes emanating from the labeled cell, consistent with the staining pattern of a plasma membrane protein. Nontransfected cells showed very little staining with MDC9-cyto IgG (Fig. 4 C), and no staining of either transfected (Fig. 4 B) or nontransfected cells (not shown) incubated with control IgG was apparent. These results suggest that, by independent assays, MDC9 is expressed at the cell surface of NIH 3T3 fibroblasts and of COS-7 cells.

**Potential Src Homology 3 Ligand Domains**

The sequence of the cytoplasmic tail of human and mouse MDC9 is relatively rich in proline residues, including two short sequences that closely resemble the consensus for Src homology 3 (SH3) ligand domains (Fig. 1 A) (2, 12, 26, 27, 38). To provide preliminary evidence that these proline-rich sequences might function as SH3 ligands, we blotted expressed gst-fusion proteins containing different portions of the MDC9 cytoplasmic tail into nitrocellulose. Gst-chimeras with the SH3 domains of Src or Abl were used to overlay blotted MDC9 gst-fusion proteins. Fig. 5, bottom panel, shows a Ponceau S stained blot of expressed MDC9 gst-fusion proteins. Fig. 5, lanes 1 and 5 contain a gst-fusion protein with both potential SH3 ligands of the MDC9 cytoplasmic tail, lanes 2 and 6 contain a gst-fusion with only the first, and lanes 3 and 7 with only the second proline-rich sequence, whereas lanes 4 and 8 contain the gst protein alone. In Fig. 5, upper panel, lanes 1–4 of the same blot are overlayed with a biotinylated Src SH3-gst-fusion protein, which bound best to the construct containing both potential SH3 ligand domains. Comparably weaker binding of Src SH3-gst to either construct with just one proline-rich sequence was evident, while Src SH3-gst did not bind to gst alone. The Ab SH3 domain, which is known to bind different ligands than the Src SH3 domain (2, 12, 26) (see below), did not bind to the MDC9 cytoplasmic gst-fusion proteins (Fig. 5, lanes 5–8, upper panel), consistent with a distinct binding specificity of the MDC9 SH3 ligand domains for different SH3 domains.

**Discussion**

The present study describes the cloning and initial biochemical characterization of the cellular disintegrin MDC9 (metalloprotease/disintegrin/cysteine-rich protein 9). Cellular disintegrins are a recently recognized protein family with at least 17 members in the mouse (16, 41, 45, 48, 49) (Weskamp, G., and C. Blobel, unpublished sequence tags), and a growing number of members in other species (4, 11,
is in a conserved position compared to related snake venom metalloproteases (6, 44). Therefore, we predict that MDC9 can function as a metalloprotease, which may be regulated through a cysteine switch mechanism (5, 6, 44). The predicted cleavage site between the MDC9 pro- and metalloprotease domain contains four consecutive arginine residues, and thus a substrate sequence for serine proteases such as the pro-protein convertase furin (10, 13, 40). Furin is known to cleave and activate another prometalloprotease, stromelysin, between the prodomain and the catalytically active domain (31).

The disintegrin domain of MDC9 is highly related to RGD-type snake venom disintegrins (6, 8, 14). An important distinction is that, like all other presently reported cellular disintegrins, MDC9 does not contain an RGD sequence in the same relative position as the snake venom disintegrin RGD. The sequence found instead of the RGD in both mouse and human MDC9 is TSE, which is directly followed by an additional cysteine residue not present in RGD-type snake venom disintegrins, but is found in all other cellular disintegrins. Several integrins are known to bind to sequences other than RGD (20), and, although the effect of the additional cysteine residue on the folding and function of cellular disintegrins remains to be established, the sequence replacing the RGD in cellular disintegrins is predicted to function as an integrin ligand (6, 8). This hypothesis is supported by fertilization studies, where pep-
tides corresponding to the predicted integrin-binding sequence of fertilin β were shown to block sperm-egg fusion, whereas scrambled control peptides did not (3, 29). Furthermore, mouse fertilization can be blocked by antibodies against the integrin α6β1 (3).

The cysteine-rich region of MDC9 that follows the disintegrin domain includes a hydrophobic sequence in the same position, relative to conserved cysteine residues, as the predicted fusion peptide of guinea pig fertilin α (8).

Like the fusion peptide of fertilin α, the hydrophobic region of MDC9 can be modeled as an amphipathic helix, which raises the question whether MDC9 could also be a membrane fusion protein. However, since expression of MDC9 is not restricted to tissues involved in cell-cell fusion, but instead is ubiquitous, any potential function of MDC9 in cell–cell fusion would have to be strictly regulated. Alternatively, this hydrophobic region may serve a different function in MDC9, such as a role in protein folding or protein–protein interactions.

The most notable feature of the cytoplasmic sequences of mouse and human MDC9 are two conserved potential SH3 ligand domains (RPPPPPQP, and RPAPAPPP), which are similar to the Src SH3-binding consensus sequence RPLPXXP (2, 12, 26). Consistent with the predicted specificity of the MDC9 SH3 ligand domains, a biotinylated Src SH3 domain interacts with the MDC9 proline-rich sequences, whereas the Ab1 SH3 domain does not (2). The binding of Src SH3 to MDC9 proline-rich sequences suggests that these may indeed function as SH3 ligand domains, although it does not imply that Src is the actual ligand in vivo, since a leucine in position 3 is found in known Src-SH3 ligand domains (2, 38). SH3 domains are present in cytoskeletal proteins and in signaling molecules (30), and it is tempting to speculate that MDC9 may interact with cytoplasmic proteins. At least two other cellular disintegrins, mouse Ms2 (49) and meltrin α (48), also contain similar cytoplasmic proline-rich sequences.

In characterizing MDC9, it was important to determine if its metalloprotease and/or disintegrin domains are membrane anchored. The functional implications are that a plasma membrane–anchored disintegrin domain could have a role in cell–cell interactions, whereas a proteolytically released disintegrin domain might function as a matrix-associated integrin ligand, or as a soluble integrin ligand which could possibly disrupt cell–cell or cell–matrix adhesion (41). Likewise, a membrane-anchored metalloprotease domain might have other roles and substrates than a soluble metalloprotease. In all mouse tissue or cell extracts that were analyzed by Western blot with MDC9-cyto IgG, the apparent molecular weight of mouse MDC9 (after removal of the signal sequence) is 89, the apparent molecular weight of 84 suggests that, in most tissues and cells, MDC9 contains a membrane-anchored metalloprotease and disintegrin domain. MDC9 could be cell surface biotinylated and 125I-iiodinated on NIH 3T3 mouse fibroblasts, indicating that MDC9 is present on the cell surface of these cells. Furthermore, the immunofluorescence staining pattern of MDC9 expressing COS-7 cells was consistent with that of a cell surface protein.

In summary, we have isolated and sequenced cDNA clones encoding mouse and human MDC9, a widely expressed cellular disintegrin. The cytoplasmic domain of MDC9 contains potential SH3 ligand domains and may play a role in interactions with the cytoskeleton or with intracellular signaling molecules. The apparent molecular weight of MDC9 in different mouse tissues and cell lines suggests that it contains both membrane-anchored disintegrin and metalloprotease domains, and might therefore have a dual function as a metalloprotease and/or integrin ligand. We anticipate that further study of this widely expressed cellular disintegrin protein will lead to a better understanding of the role of members of this newly recognized protein family in cell–cell interactions.

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