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MN-cadherin and its novel variant are transiently expressed in chick embryo spinal cord

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Abstract

To isolate cDNAs that are involved in limb-motoneuron development, we compared mRNAs of lumbar and thoracic motoneurons purified from spinal cord of E4 chick embryo by differential display. In situ hybridization demonstrated that one of cDNAs is expressed exclusively in lateral motor column in spinal cord from E4 to E10. We identified two mRNA variants for the cDNA by library screening. The long form (788 amino acids) was identical to chick MN-cadherin. The short variant (543 amino acids) lacks the first two of five extracellular domains of MN-cadherin, which commonly exist in classical cadherins. The amino acid sequence of the short form is identical to that of the carboxyl terminal MN-cadherin, except for the distinct signal sequence. The ratio of mRNA of short form to long form was 1–20. cDNA transfection study revealed that the long form but not the short form MN-cadherin had cell adhesion activity.

Keywords: Motoneuron; Chick embryo; MN-cadherin; Variant; Cell adhesion

Wiring an enormous number of neurons in highly precise pattern is essential for the formation of the complex architecture of vertebrate nervous system. The formation of neural network primarily depends on the ability of growth cone to interpret environmental cues along the pathway to the target region. Recent discovery of extracellular signaling molecules which act as chemoattractive cue, Netrin, and chemorepulsive cue, Semaphorin and Slit, and the receptors (DCC, Eph, Robo, Plexin, and Neuropilin) has lead us to understanding of basic aspects of molecular mechanism of network [1,2], although it is not yet complete because of high degree of complexity of the nervous system.

Chick embryonic motoneuron in the spinal cord has been one of the model systems to study the mechanism of development of specific neural network [3]. Motoneurons are divided into two groups of cells, medial motor column (MMC) and lateral motor column (LMC). MMC motoneurons innervate axial muscles and exist in the medial part of the motor column in all levels of the spinal cord along anterior–posterior axis. LMC motoneurons innervate limb muscles and reside in lateral to the MMC in the levels of brachial and lumbar positions in the spinal cord. LMC motoneurons innervating the hind limb are composed of more than 20 distinct subtypes that innervate specific target muscles [4]. Axons of those neurons enter and grow to the correct direction in the plexus, select correct pathways at choice points, and innervate appropriate target muscles,

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suggesting that each subtype express distinct molecule(s) for pathfinding that are not yet identified.

In an attempt to isolate molecules that are involved in developmental events in LMC motoneurons, we performed differential display comparing mRNA from motor neurons isolated from cervical, thoracic, and lumbar spinal cord of E4 chick embryo and reported CEPU-1 as one of the molecules expressed in developmentally regulated manner in the motor column [5]. In this study, we report isolation of MN-cadherin [6] (long form) and its novel (short) variant that are expressed in the lateral motor column in the period of axonogenesis of motoneurons. The short variant lacks two NH_2-terminal domains, EC1 and EC2, which have been shown to be involved in cell adhesion activity [7,8]. Characterization of the cells that express either long form or short form showed that long form MN-cadherin has calcium-dependent cell adhesion activity but short form does not, while interaction with β-catenin was suggested for both forms of MN-cadherins. Lack of cell adhesion activity of sMN-cadherin clearly indicates importance of EC1 and/or EC2 domains in adhesion activity.

Materials and methods

Animals. Fertilized eggs of White Leghorn chicken were purchased from commercial sources. They were incubated at 38 °C. Staging of the embryo was made according to Hamburger and Hamilton [9].

RNA preparation. Motoneurons were purified separately from cervical, thoracic, and lumbar portion of the spinal cord of 4E chick embryo by panning method utilizing anti-SC1 monoclonal antibody that recognizes motoneuron and floor plate cells in the spinal cord in the period of 4E–7E [10]. The cells purified by this method contain motoneurons at approximately 80%. Total RNA for differential display was isolated from motoneurons derived from each portion of the spinal cord with conventional method [11] and treated with RNase-free DNase I (Gibco-BRL) according to the manufacturer's instruction. Total RNA for competitive PCR-analysis was isolated from spinal cords of 4E–8E embryos.

Differential display. Differential display was performed as described previously except that the primers were labeled ^{32}P in this study instead of fluorescence [12]. The RNAs (0.6 μg) from cervical, thoracic, and lumbar motoneurons were converted to cDNA by M-MLV reverse transcriptase (Gibco-BRL) with 30 pmol of 5′-CCCGGATCCTTTTT TTTTTTTTTC-3′ (C-anchor primer). cDNA synthesized from 8 ng of total RNA was amplified with 5′-TCCGATGGTACCTAAATTAGC-3′ and ^{32}P-labeled C-anchor primer. The cycling parameters were as follows: the first cycle was 94 °C for 40 s, 37 °C for 5 min, 72 °C for 2 min and it was followed by 30 cycles of 94 °C for 40 s, 55 °C for 1 min, and 72 °C for 2 min. Amplified cDNA was subjected to electrophoresis on 6% long ranger gel (FMC Bioproducts) containing 8.3 M urea and visualized by autoradiography. The band that showed intense signal in lumbar cDNA (Fig. 1) was excised from the gel and DNA was recovered by heating the gel at 100 °C for 5 min in 100 μl of H_2O. The DNA was reamplified by 30 cycles of PCR with the same combination of the primers used for differential display. Reamplified cDNA was cloned into pCR II cloning vector (Invitrogen).

Screening and sequencing of MN-cadherin cDNA. The cDNA library constructed from poly(A)$^+$ RNA of 14E chick spinal cord at lumbar level in lambda-Zap vector (Stratagene) [13] was screened by ^{32}P-labeled X37 DNA (375 bp) as a probe.

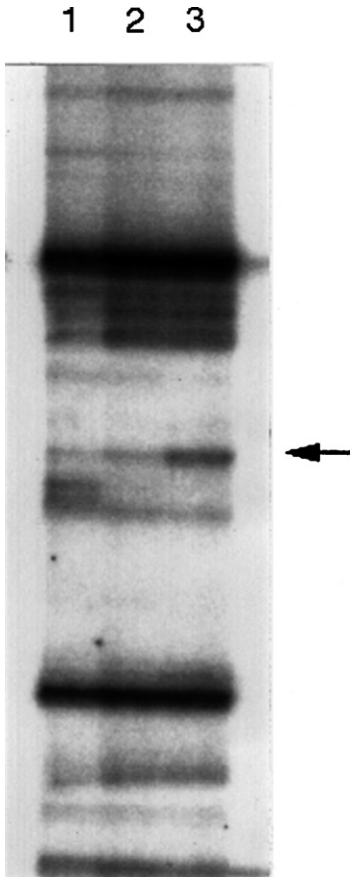

Fig. 1. Differential display analysis of RNA of motoneurons from different position of 4E chick embryo spinal cord. RNAs from cervical (lane 1), thoracic (lane 2), and lumbar motoneurons (lane 3) were reverse-transcribed to cDNA with oligo(dT) primer. Each cDNA was PCR-amplified with ^{32}P-labeled oligo(dT) and arbitrary primer under non-stringent conditions (see Materials and methods). PCR products were subjected to 6% polyacrylamide gel. The arrow indicates 370 bp band with higher intensity in lumbar cDNA.

In situ hybridization. Anti-sense riboprobe to detect both long and short form of MN-cadherin was generated by transcribing *Eco*RI-linearized full-length cDNA of the long form of MN-cadherin in pBluescript SK- with T3 RNA polymerase (Gibco-BRL) in the presence of 0.4 mM digoxigenin-11-UTP (Roche Molecular Biochemicals) for the synthesis of sense probe, the plasmid digested with *Xho*I was transcribed with T7 RNA polymerase (Gibco-BRL). In situ hybridization of frozen section was performed as described previously [14].

Competitive RT-PCR. Total RNA (5 μg) prepared from embryos of 4E–7E was converted to cDNA by the reaction of M-MLV reverse transcriptase (Gibco-BRL, 200 U) at 16 °C for 10 min and at 42 °C for 90 min in the 20 μl of reaction mixture (50 mM Tris–HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 20 mM dithiothreitol) containing 50 ng of random hexanucleotide primer (Roche Molecular Biochemicals), 2 U of human placental RNase inhibitor (Takara), and 0.5 mM dNTP. Aliquots (150) were PCR-amplified by hot start PCR using *Taq* DNA polymerase (Gibco-BRL) bound to TaqStart antibody (Clontech) with primer LF1 (forward, 5′-GATGGACCTAACTACAACT GTGCT-3′) and primer LR1 (reverse, 5′-CTGTGGAAATTGGGTG GGTTGTC-3′) for the detection of the long isoform and with primer SF1 (forward, 5′-GGTGCTCACATTTGTGTGTTTATC-3′) and SR1 (reverse, 5′-CTGTGGAAATCTGGGTGGGTTGTC-3′) for the

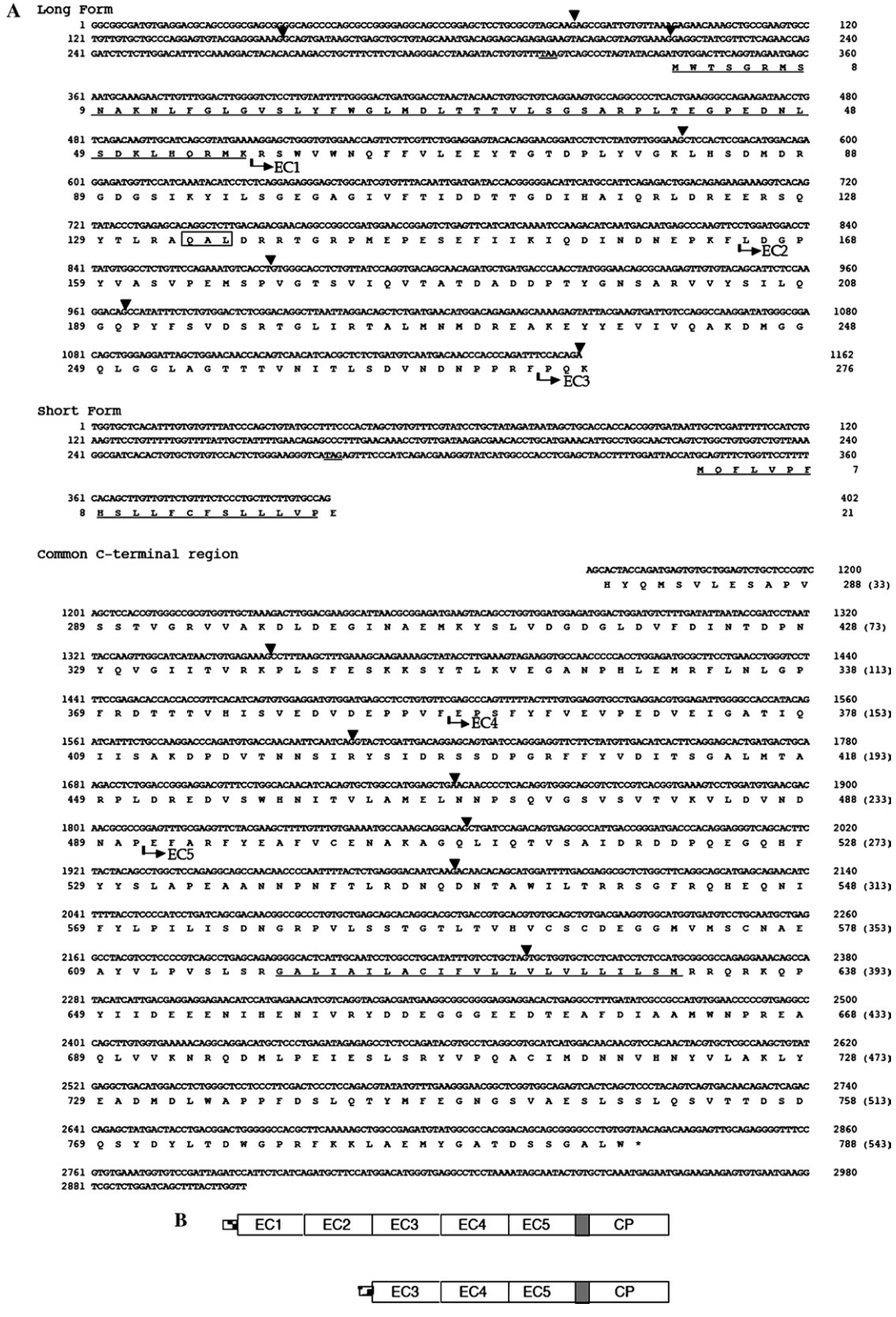

Fig. 2. Primary structure of MN-cadherin and short isoform sMN-cadherin. (A) Nucleotide sequence and deduced amino acid sequence of MN-cadherin and sMN-cadherin. Nucleotide and amino acid residues are numbered on both sides. The numbers in the parenthesis are for C-terminal portion of sMN-cadherin. The putative signal sequence is underlined. The position of the intron is indicated by arrowhead. In-frame stop codons upstream to the initiation codon are underlined. Tripeptide that is conserved in type II cadherins is boxed. Potential N-glycosylation sites are double-underlined. Transmembrane segment is underlined. (B) Schematic drawing of MN- and sMN-cadherins. Hatched boxes represent signal peptide. Shadowed boxes designate transmembrane segments.

detection of the short isoform in the presence of various concentrations of respective competitive DNA to estimate the content of the cDNA under the following conditions: 94 °C for 40 s, 56 °C for 1 min, and 72 °C for 2 min for 35 cycles. Heterologous DNA competitors with the respective primer sequences at the both ends of the fragments were generated by using Competitive DNA construction kit (Takara). Competitor DNA was diluted with 10 mM Tris–HCl (pH 8.0), 1 mM EDTA containing 0.1 mg/ml yeast RNA (Roche Molecular Biochemicals). A series of PCR containing two-fold serially diluted competitor DNA (ranging from 1.4×10^6 to 4.4×10^4 copies for long isoform, 5×10^4 to 1.6×10^3 for short isoform) were added to PCR. The amount of cDNA of two isoforms in different developmental stage was estimated by calculating number of cDNA per microgram of RNA used for RT-PCR as reported previously [15].

Establishment of monoclonal antibody for MN-cadherin. The cDNA region encoding EC3 and EC4 of MN-cadherin (amino acids 288–491) was PCR-amplified and subcloned into polylinker of pGEX-1 vector (Pharmacia) to express these domains in *Escherichia coli* as a fusion protein (EC3, 4-GST) to glutathione *S*-transferase (GST). Expression of the fusion protein was induced by the addition of 1 mM isopropyl-β-D-thiogalactoside to the culture medium. The fusion protein was purified from the soluble fraction as suggested by manufacturer's using GST purification module (Pharmacia). Balb/c mice were immunized three times with EC3, 4-GST (100 μg) at 2 weeks intervals. Dispersed spleen cells from the animal were fused with 653 myeloma cells and hybridoma screening was performed as described by Köhler and Milstein [16].

Immunohistochemistry. Immunohistochemistry was performed according to the protocol of TSA-kit (NEN Life Science Products). The embyro was fixed with 4% paraformaldehyde. The signal generated by peroxides-Tyramide-FITC was observed on Nikon ECLIPSE E600 fluorescence microscopy. Antibody to O4 antigen [17] was generous gift from Dr. Ono of Shimane Medical University.

Establishment of cell lines expressing long and short forms of MN-cadherin. The expression vectors pUHG-MNL and pUHG-MNS were constructed to express long and short form of MN-cadherins by cloning cDNAs to the downstream of CMV minimal promoter on pUHG 10-3 vector, which can be activated by tTA (tetracycline-controlled transactivator) upon removal of tetracycline [18]. After ligation of *Eco*RI–*Xho*I fragment containing either long form or short form with *Eco*RI and *Xba*I digested pUHG 10-3 vector, *Xho*I and *Xba*I sites were blunt-ended by the treatment of Klenow fragment (Takara), followed by ligation again to circularize the vector. Transfection of the expression vectors into L cells was performed using LipofectAMINE reagent (Life Technologies) together with pTA-Hyg that express tTA transactivator and confer hygromycin resistance to the cells, according to the manufacturer's instructions. The transfected cells were selected in an 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium supplemented with 10% fetal bovine serum in the presence of 200 μg/ml of hygromycin B (Wako Pure Chemical Industries) and 2 μg/ml tetracycline (Wako Pure Chemical Industries) in humidified atmosphere comprising 5% CO_2, 95% air at 37 °C for about 2 weeks. Then hygromycin resistant colonies were isolated, screened for MN-cadherin expression after induction by removal of tetracycline and maintained under the same conditions in the presence of tetracycline.

Cell aggregation assay. Dispersed cell suspension was obtained by treating cells with 5 mM Hepes-buffered calcium and magnesium-free Hank's solution (HCMF) containing 0.001% trypsin (Sigma T8003) and 1 mM $CaCl_2$ at 37 °C for 20 min at 80 rpm. Then they were washed three times with HCMF and suspended at 10^5 cell particles/ml in HCMF containing 1% bovine serum albumin with or without 1 mM $CaCl_2$. The cell suspension (0.5 ml) was dispensed into bovine serum albumin-coated 24-well plastic plate (NUNCLON Surface; Nalgen Nunc International). After incubating at 37 °C at 80 rpm, the cells were gently pipetted and cell particles were counted at the times indicated in Fig. 7.

Results and discussion

cDNA cloning of MN-cadherin cDNA

As described previously [5] we performed differential display in order to identify mRNA that are specifically

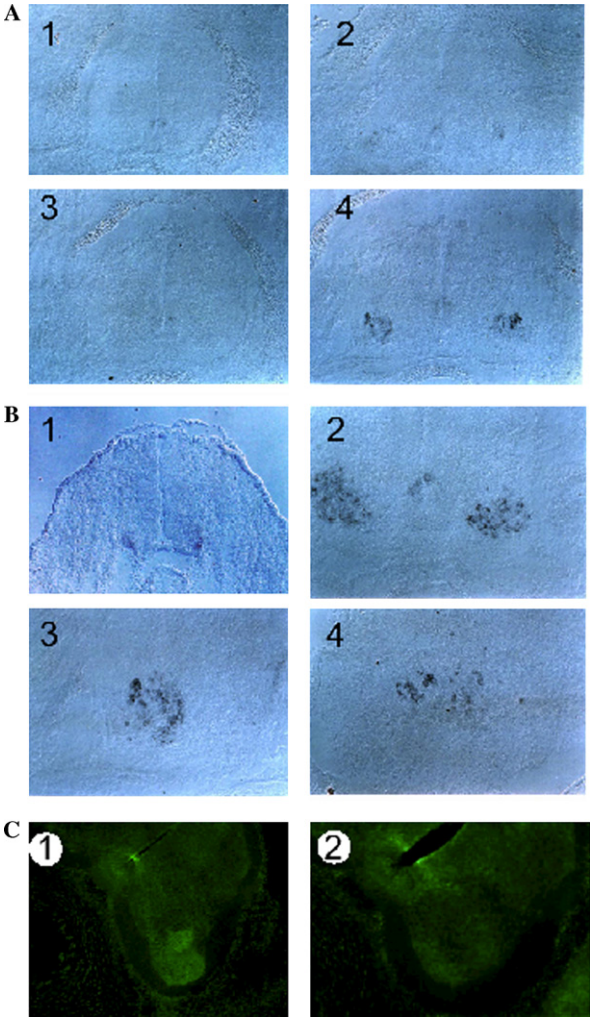

Fig. 3. Expression of MN-cadherin in chick embryonic spinal cord by in situ hybridization and immunostaining. (A) Transverse sections of chick embryo at cervical position (1), brachial position (2), thoracic position (3), and lumbar position (4) at stage 36 were hybridized with digoxygenin-labeled riboprobe. The signals were detected in the motor column only in brachial and lumbar sections. Small number of cells above the floor plate are stained. (B) In situ hybridization of transverse sections at lumbar position at stages 23 (1), 29 (2), 32 (3), and 36 (4). The signal was distributed diffusely in motor column at stage 23, but become restricted to part of motor column at later stages. Immunostaining of transverse section of chick embryo at lumbar position (C1) and thoracic position (C2) at stage 32. Motor column at lumbar but not thoracic position was stained. C2, no signal was observed in the motor column at thoracic position. Signal was observed in small number of cells above the floor plate at both positions.

expressed in motoneurons at lumbar region. Motoneurons were purified by panning method with anti-SC1 antibody from the spinal cord excised from 4E chick embryo separately at cervical, thoracic, and lumbar level. Total RNA from each motoneuron pool was reverse-transcribed and PCR-amplified with ^{32}P-labeled 3′-anchor primer under the conditions described in the experimental procedures. The PCR products were subjected to gel electrophoresis and visualized (Fig. 1). The band (X37), with stronger signal in lumbar mRNA than in cervical and thoracic mRNA, was recovered from the gel and PCR-amplified with the same primers used for the differential display. To isolate full-length cDNA of X37, we screened cDNA library from 14E chick embryo spinal cord excised from lumbar level. Twelve clones, which include two kinds of cDNA, were isolated from 7×10^5 plaques. Eleven of twelve clones had common physical map and contained open reading frame of 2364 bp coding for 788 amino acids that is identical to MN-cadherin [6] reported as type II cadherin expressed in the subset of motoneruons and functions in segregating the motoneruon pool through its cell adhesive activity (Fig. 2A). Nucleotide sequence of X37 cDNA fragment isolated by differential display agreed with that of 3′-noncoding regions of the clones. One of the cDNAs contained an open reading frame of 1629 bp coding for 543 amino acids. The deduced amino acid sequence indicated that the cDNA encodes an alternative form (short form) cDNA. Although only one cDNA was isolated for the short form by library screening, presence of this variant mRNA was confirmed by RT-PCR analysis (see below).

DNA sequence of the short isoform (sMN-cadherin) was completely coincided with the sequence downstream from the position 1165 nucleotide of MN-cadherin cDNA including 436 bp 3′-noncoding region, suggesting that these forms are transcribed from the single gene. sMN-cadherin has putative signal peptide composed of 21 amino acids and 5′-non-coding region distinctive from that of MN-cadherin. BLAST search of while chick genome sequence revealed that sMN-cadherin-specific sequence coding for signal sequence was found in the 5′ to exon 8 of MN-cadherin, suggesting that the mRNA for sMN-cadherin might be transcribed from alternative promoter that exists in the intron between EC2 and EC3 coding exons of MN-cadherin.

Expression of MN-cadherin in spinal cord by in situ hybridization and immunohistochemical analysis

Expression of MN-cadherin in spinal cord during the period of axonal growth of spinal motoneuron was examined by in situ hybridization of transverse section of the spinal cord at stage 21, 23, 29, 32, and 36 with a probe common to both MN-cadherins. From stage 23 to 36, MN-cadherin mRNA was expressed in the motor column that is located at ventro-lateral portion of spinal cord (representative data at stage 36 was shown in Fig. 3A). The expression in the motor column was detected in the brachial and lumbar positions but not

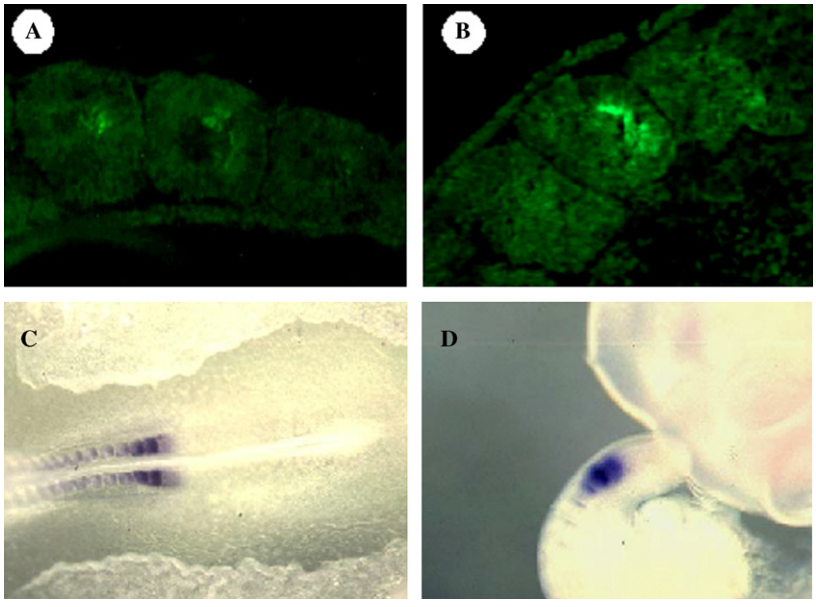

Fig. 4. Expression of MN-cadherin in newly generated somites shown by in situ hybridization and immunohistochemical analysis. Sagital section of somites at stage 18 (A) and 24 (B) were stained by anti-MN-cadherin monoclonal antibody. Signals were observed in the caudal half of newly generated somites and presomitic mesoderms at stages 18 and 24. Embryos at stage 18 (C) and 24 (D) were hybridized with digoxygenin-labeled riboprobe. Signals were observed in the caudal half of newly generated somites and presomitic mesoderms.

in the cervical and thoracic positions of the spinal cord in agreement with the result of differential display. These results indicate that MN-cadherin was expressed in the lateral motor column, on which the motor neurons innervate limb muscles in agreement of the report by Price et al. [6].

Expression of MN-cadherin protein was examined by monoclonal antibody staining. Transverse sections at various positions of spinal cord from E2 to E9 embryo were examined. Motor column at lumbar (Fig. 3B2) and brachial (not shown) position was most intensely stained at stage 32. No signal was observed in the motor column at thoracic position (Fig. 3B1), suggesting that MN-cadherin protein is expressed in the lateral motor column.

A small group of cells above the floor plate was stained with the antibody (Figs. 3B1 and B2). The signal was observed throughout the spinal cord in contrast to the case of motor neurons. Similar expression pattern was reported for O4 antigen that is a specific marker for precursors of oligodendrocytes [17]. We stained sequential transverse sections of the spinal cord with anti-MN-cadherin and anti-O4 antibody. The cells stained with anti-MN-cadherin were located in between the floor plate and O4-positive cells (data not shown). Identification of MN-cadherin-positive cells above the floor plate, therefore, has to await further experiments.

MN-cadherin expression in other tissues

We next carried out whole mount in situ hybridization to examine the expression of the gene in whole embryo. Signals were observed in the caudal half of newly generated somites and presomitic mesoderms at stages 18 and 24 (Figs. 4A and B, respectively). Immunohistochemical analysis also detected expression of MN-cadherin in the apical end of cells surrounding the central core of the newly formed somites. The fact that only a trace amount of mRNA and protein remained in the next somites to the newest ones suggests short half life

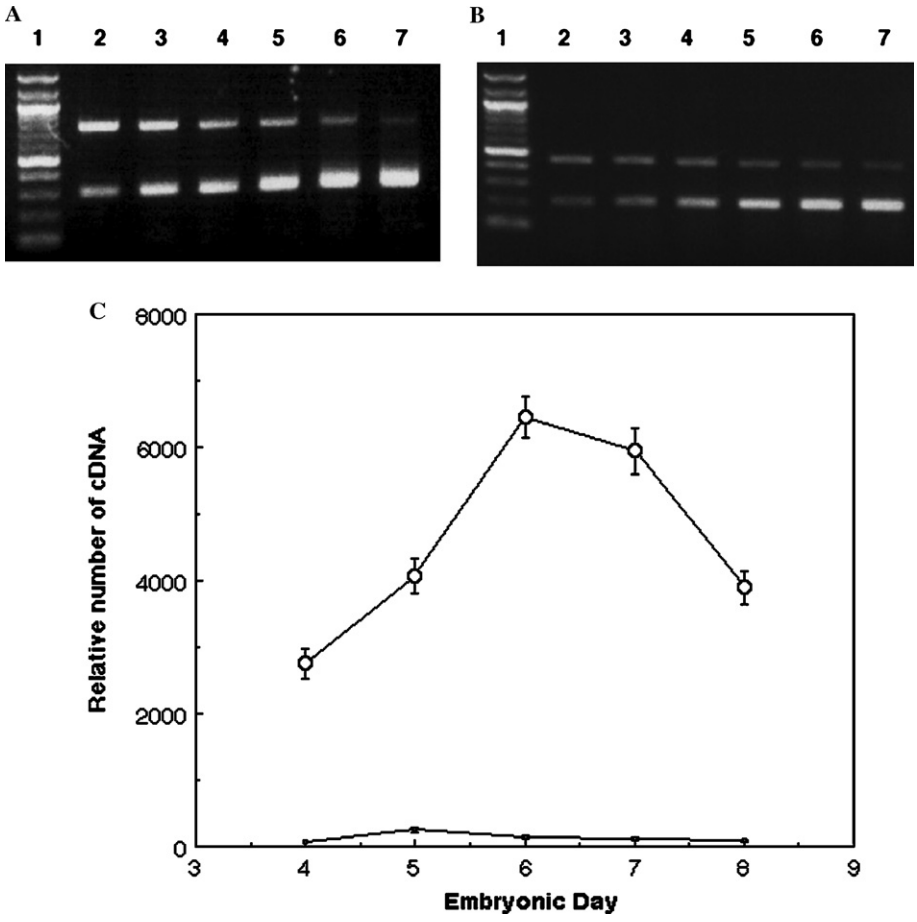

Fig. 5. Estimation of expression level of MN- and sMN-cadherins by RT-PCR. (A) cDNA prepared from E5 chick embryonic spinal cord was PCR-amplified with primers LF1 and LR1 in the presence of 4.4×10^4 (lane 2), 8.8×10^4 (lane 3), 1.75×10^5 (lane 4), 3.5×10^5 (lane 5), 7×10^5 (lane 6), and 1.4×10^6 (lane 7) copies of competitor DNA. (B) cDNA prepared from E5 chick embryo spinal cord was PCR-amplified with primers SF1 and SR1 in the presence of 1.6×10^3 (lane 2), 3.1×10^3 (lane 3), 6.25×10^3 (lane 4), 1.25×10^4 (lane 5), 2.5×10^4 (lane 6), and 5×10^4 (lane 7) of copies of competitor DNA. (C) Expression level of MN-cadherin (○) and sMN-cadherin (□) during embryogenesis of chick spinal cord. Maximum expression of MN-cadherin and sMN-cadherin were at E6 and E5, respectively.

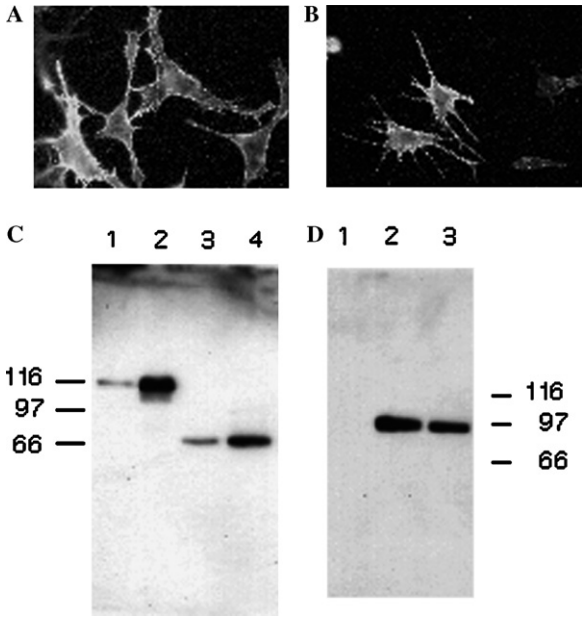

Fig. 6. Establishment of stable cell lines expressing MN- and sMN-cadherins. (A) L cells expressing MN-cadherin (LL2221) by removal of tetracycline were stained with anti-MN-cadherin antibody without fixing the cells. (B) L cells expressing sMN-cadherin (LS4822) were stained with anti-MN-cadherin antibody. (C) Western blotting analysis of cell extracts of LL2221 in the presence (lane 1) and absence (lane 2) of tetracycline and LS4822 in the presence (lane 3) and absence (lane 4) of tetracycline. (D) Western blotting with anti-β-catenin antibody. In L cell (lane 1) β-catenin was not detected because of instability in the absence of cadherin. Both MN-cadherin (lane 2) and sMN-cadherin (lane 3) stabilized β-catenin.

of MN-cadherin mRNA and protein, considering that new somites are formed in every 90 min in chick embryo [19]. N-cadherin and cadherin-11 are expressed in the luminal side of somites. In double mutant mice lacking both N-cadherin and cadherin-11, somites were fragmented into rostral and caudal halves and form separated aggregation, suggesting the presence of distinctive cell adhesion molecules in both halves of somites that function in maintaining rostral and caudal identity of somitic cells. Our data suggest that MN-cadherin might be a good candidate for the cell adhesion molecule that aggregates the caudal half of the somites.

Relative amount of long and short isoforms by competitive RT-PCR

Estimation of relative amount of short and long MN-cadherin isoforms was performed by competitive RT-PCR method. Long and short forms of MN-cadherins were separately detected by specific primers (Figs. 4A and B). Internal control was generated by linking respective primer sequences to 5′ and 3′ of λ phage DNA as described under Materials and methods. Total RNA isolated from various stages of chick embryo spinal cord was converted to cDNA and

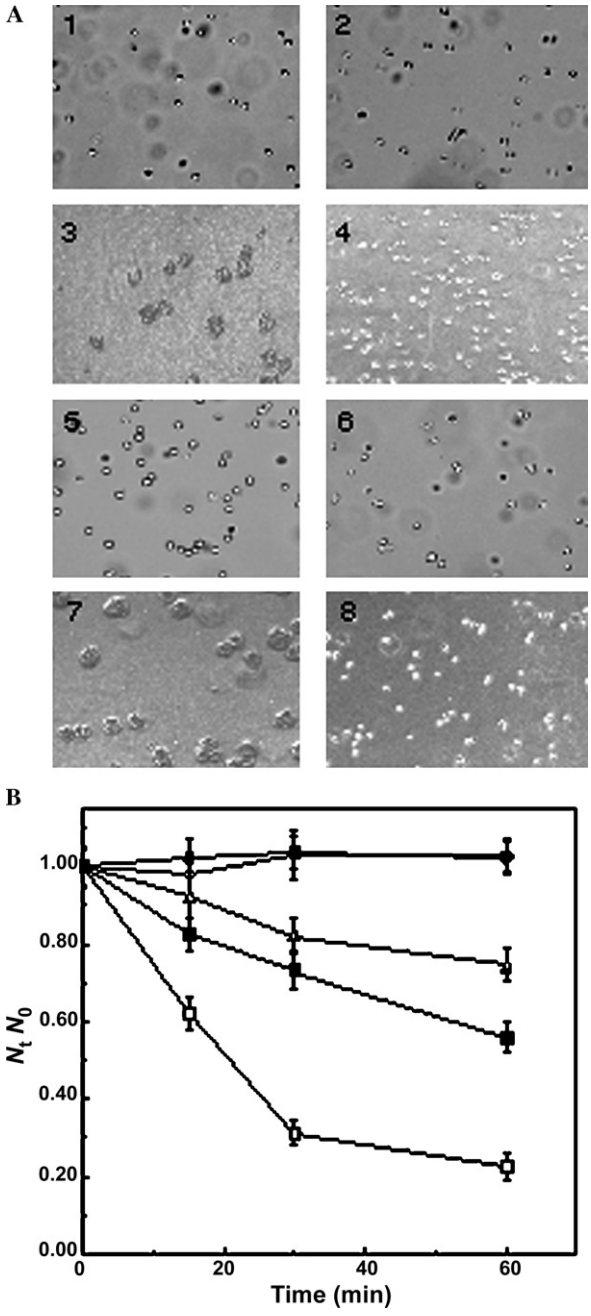

Fig. 7. Aggregation assay of MN-cadherin or sMN-cadherin positive cells. (A) After dissociation of parental L cells and MN-cadherin, and sMN-cadherin expressing cell lines,the cells were allowed to aggregate at 80 rpm for 60 min and analyzed. L cells did not form any aggregates both in the presence and the absence of calcium (A1 and A2). MN-cadherin expressing cell line form small aggregates in the presence (A3) but not in the absence of calcium (A4). sMN-cadherin expressing cells did not form any aggregates both in the presence (A5) and absence of (A6) calcium. Cadherin-11 expressing cell line form small aggregates in the presence (A7) but not in the absence (A8) of calcium. (B) Kinetics of aggregation. The values are the means of three independent experiments. N_t and N_0 are the total number of particles at incubation times t and 0, respectively. The aggregation is represented by N_t/N_0 index. Open circle, close circle, open triangle, close box, and open box designate L cells, sMN-cadherin expressing cell line, MN-cadherin expressing cell line, cadherin-11 expressing cell line, and N-cadherin expressing cell line, respectively.

PCRs were carried out after adding seriously diluted competitive DNA to each reaction. Ratios of long and short MN-cadherin were estimated by calculating the amount of cDNAs in the reaction. Maxima of expressions were at E6 and E5 for long and short MN-cadherins, respectively (Fig. 5). The ratio of short form to long form was the highest at E5 and the value was one-twentieth.

Cell adhesion activity of authentic and variant forms of MN-cadherin

To characterize the adhesive activity of long and short form cadherins, both forms of MN-cadherins were expressed under the control of tetracycline-responsive promoter in mouse L cell. L cell is widely used to examine cadherin-mediated cell adhesion, since it lacks its own cadherin but express β-catenin that is essential for cadherin-mediated cell adhesion [20]. MN-cadherin and sMN-cadherin cDNAs were cloned onto downstream of the CMV promoter, which can be activated by tTA (tetracycline-controlled transactivator) in the absence of tetracycline, in the pUHG-10- [18] vector to generate pUHG-LF and pUHG-SF expression vectors, respectively. Expression vectors were co-transfected with pTA-Hyg that provide with the activator and confer hygromycin resistance on the cells. Transfected L cells were selected with hygromycin and the expression of the proteins was examined by immunostaining with 18E6 monoclonal antibody after induction upon removal of tetracycline from the culture medium. Two lines (LL2221 and LL2219) expressing MN-cadherin and two lines (LS4822 and LS4833) expressing sMN-cadherin were obtained. LL2221 and LS4822, which showed stronger expression, were used for further analysis. Positive immunostaining of LL2221 and LS4822 without fixation (Fig. 6) suggests that both proteins were displayed on the cell surface. Western blotting analysis of LL2221 and LS4822 cells identified a band with 115 kDa of molecular mass and a band with 75 kDa molecular mass, respectively in tetracycline-induced cells (Fig. 6C). Introduction of long and short form of MN-cadherin into L cells increased the level of β-catenin (Fig. 6D) as reported for classical cadherin [20], suggesting that both long and short form MN-cadherins interact with subcellular machinery for cadherin-mediated cell adhesion.

Cell adhesion activity of long and short form of MN-cadherin

Cell aggregation assay was carried out by the established method of [21] for LL2221 and LS4822 cell lines to examine adhesion activity of long and short form MN-cadherins. Parental L cells, L cells expressing N-cadherin (type I classical cadherin) and L cell expressing cadherin-11 (type II classical cadherin) were also examined for comparison. In the cell aggregation assay, N-cadherin transfectants began to aggregate within 15 min of rotation (Fig. 7B) and form large aggregates after 1 h (data not shown). Cadherin-11 transfectants and LMN-cadherin transfectants made relatively small aggregates in Ca^{2+}-dependent manner (Figs. 7A3, A4, A7, and A8). On the other hand, sMN-cadherin transfectants (Figs. 7A5 and A6), as well as parental L cells did not form any aggregates. These results indicate that MN-cadherin has significant cell adhesion activity comparable to cadherin-11 that belong to type II classical cadherin, but has weaker activity, comparing with N-cadherin (one of type I classical cadherins) (Fig. 7B).

In conclusion, we cloned chick MN-cadherin and its novel variant expressed in LMC and newly generated somites. Expressions of two forms of cadherins are differentially regulated. Long form but not short form showed cell adhesive activity. Since dominant negative N-cadherin lacking extracellular domain was shown to inhibit cell adhesion of authentic cadherin in vitro [22] and affect retinal neuron guidance in vivo [23], sMN-cadherin may have distinct function from authentic MN-cadherin that remains to be determined.

Acknowledgments

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