Smooth muscle type isoform of 20 kDa myosin light chain is expressed in monocyte/macrophage cell lineage

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Abstract

Myosin light chain genes of human hematopoietic cells have not been fully characterized. We previously reported the cloning of the full-length cDNAs of 20 kDa regulatory myosin light chain (MLC-2), named as MLC-2A, from Meg-01, a human megakaryoblastic leukemia cell line (*J. Smooth Muscle Res.* 37: 25–38, 2001). We now cloned another MLC-2 isoforms from human platelets and U937, a human monocytic leukemia cell line, named as MLC-2B and MLC-2C, respectively. Both MLC-2A and MLC-2B consisted of three exons, which were situated on gene loci 18p1.3. Analysis of the gene structure indicated that MLC-2A and MLC-2B utilized different exons. MLC-2C also consisted of three exons, which was situated on gene loci 20p12. Amino acid sequence of MLC-2C was, of interest, apparently almost the same as that of MLC-2 from chicken gizzard smooth muscle LC20-A (one amino acid's difference) and human vascular smooth muscle LC-20 (two amino acids' difference). All three protein kinase C phosphorylation residues (Ser-1, Ser-2, Thr-9) and both myosin light chain kinase phosphorylation residues (Thr-18, Ser-19) are conserved in these three isoforms. The MLC-2A and MLC-2B mRNA were expressed constitutively in all of the human hematopoietic cell lines examined and their expression levels were almost the same. On the other hand, MLC-2C mRNA was expressed in untreated monocytic cell lines (U937 and A-THP-1) and HL-60 differentiated into monocyte/macrophage cell lineage by TPA treatment. These results indicate that smooth muscle type isoform, MLC-2C is the inducible isoform, and might play a crucial role in monocyte/macrophage cell lineage.

Key words: myosin, myosin light chain, isoform, hematopoietic cell

Introduction

Two major contractile proteins actin and myosin are present in almost all eukaryotic cells. Evidence has emerged for the existence of a large superfamily of myosins. To date, 18 structurally distinct classes of myosins have been identified (Foth *et al.*, 2006). In conventional

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myosin (myosin II), myosin filaments share the same architecture of two identical 200 kDa myosin heavy chains (MHCs) and two pairs of myosin light chains (MLCs). Two of these light chains are classified as a phosphorylatable regulatory chain of 20 kDa (MLC-2) and a nonphosphorylatable essential chain (MLC-1 or MLC-3) of 17 kDa (Harrington *et al.*, 1984). MLC-2 is phosphorylated by myosin light chain kinase (MLCK) in the presence of calcium and calmodulin and increase actin-activated ATPase activities of myosins of both smooth muscle (Adelstein *et al.*, 1984; Somlyo *et al.*, 1994) and nonmuscle cells such as platelets (Ikebe, 1989; Higashihara *et al.*, 1991). Phosphorylation of MLC-2 initiates the contraction of vertebrate smooth muscle (Hartshorne *et al.*, 1982), and is presumed to play a similar role in functions of nonmuscle cells (Ikebe, 1989; Higashihara *et al.*, 1991). MLC-2 phosphorylation of cytoplasmic myosin has been implicated in cytokinesis, receptor capping, and cell locomotion (Bourguignon *et al.*, 1981; Berlot *et al.*, 1985; Yamakita *et al.*, 1994; Matsumura *et al.*, 1998).

There are several reports, suggesting the existence of MLC-2 isoforms. We previously isolated MLC-2 from bovine red blood cells, showing that whose molecular weight is 19.5-kDa (Higashihara *et al.*, 1989), in contrast to 20-kDa of human red blood cells (Fowler *et al.*, 1986; Higashihara *et al.*, 1989). Myosins from rabbit alveolar macrophages are heterogeneous with respect to its MLC-2, showing that two species of MLC-2 are identified by one-dimensional and two-dimensional polyacrilamide gel electrophoresis, in a ration of 2:1. Native myosin, analyzed on non-denaturing gels, is also composed of two species, in a ration of 2:1 (Trotter *et al.*, 1983). Isoform of alkali-light chain was reported in skeletal muscles (Lowey *et al.*, 1979). Myosins from myeloid leukemia cells are heterogeneous with respect to MLC-2 and both variants are phosphorylated by endogenous MLCK (Sagara *et al.*, 1982a; Sagara *et al.*, 1982b). Membrane-associated and cytoplasmic myosins from platelets probably contain different heavy chains (Peleg *et al.*, 1983; Peleg *et al.*, 1984). Bovine thymus lymphocyte was reported to have two to three MLC-2 isoforms which migrate with differently charged species on two-dimensional gels (Scholey *et al.*, 1982). Fechheimer *et al.* (1982) reported an unidentified, phosphorylated polypeptide with a molecular mass of 22,000 daltons is co-isolated with myosin by rapid immunoprecipitation. Kumar *et al.* (1989) reported the analysis of the MLC-2 immunoprecipitates using two-dimensional gel electrophoresis and confirmed that there are three MLC-2 isoforms in human nonmuscle cell lines, and that some nonmuscle cell lines such as human lung fibroblasts, foreskin fibroblasts express on one smooth muscle and two nonmuscle isoforms, whereas T cells express only two nonmuscle isoforms.

We previously reported on sequence analysis of cloned cDNAs of MLC-2 and 17 kDa essential myosin light chain (MLC-3) from the human megakaryoblastic leukemia cell line, Meg-01 (Watanabe *et al.*, 2001). This was apparently the first documentation of cloning of full-length cDNA of MLC-2 from human nonmuscle cells. The similarity between the deduced amino acid sequences of Meg-01 and lymphocyte (GenBank U26162) is 100%. We now report the second and third type of MLC-2 isoform, and the latter was, of interest, apparently almost the same as the sequence of MLC-2 from chicken gizzard smooth muscle.

Methods

Cell lines and cell cultures

Reh, NC37, Jurkat, U937, HEL, Meg-01 and CMK cell lines were obtained from the Japanese Cancer Research Resources (JCRB) Cell Bank (Tokyo, Japan). U266 cell line was obtained from Hayashibara Biochemical Laboratories, INC., Fujisaki Cell Center (Okayama, Japan). NALM-6, Daudi, Raji, RPMI 8226, HUT78, HL-60, TPH-1, A-TPH-1 and K562 cell lines were obtained from Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer Tohoku University (Sendai, Japan). All cell lines were cultured in RPMI 1640 containing 10% (v/v) fetal calf serum (FCS), 100 units/ml penicillin and 100 μ g/ml streptomycin. Cord bloods were obtained from healthy volunteers. Mononuclear cells were separated by Lymphoprep (Nycomed Pharma AS, Oslo, Norway) according to manufacturer's instructions.

Human platelets were obtained from whole blood anticoagulated with acid-citrated dextrose (ACD) solution (blood : ACD=6 : 1, v/v) from healthy volunteers. Whole blood was centrifuged at 1,000 rpm, 10 min, and platelet-rich plasma (PRP) was filtered through the Sepharose 2B column to remove the white blood cells. Filtrated PRP was centrifuged at 1,000 rpm, 5 min to remove the residual contaminated white blood cells and red blood cells. The supernatant was used the source of RNA from human platelets.

Chemical

12-O-tetradecanoylphorbol-13 acetate (TPA) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved with ethanol.

Treatment of the HL-60 cells with TPA

HL-60 cells were seeded at a density of 3×10^5 cells/ml and treated with 20 nM of TPA for indicated hours. Adhesive cells were detached by using Trypsin-EDTA (Sigma-Aldrich, St. Louis, MO, USA).

Total RNA extraction and purification

Total RNA was extracted using ISOGENTM (Nippon Gene, Co., Ltd, Tokyo, Japan). Poly (A)⁺ RNA was purified using OligotexTM dT-30 super (Takara Shuzo, Kyoto, Japan) according to the manufacturer's instruction.

Oligonucleotides and polymerase chain reaction (PCR)

Oligonucleotides were purchased from ESPEC OLIGO SERVICE (Tsukuba, Japan). For PCR we used Takara TaqTM DNA polymerase (Takara Shuzo, Kyoto, Japan) and standard PCR reaction mixtures with 25 pmole each of forward and reverse primers in a 50 μ l cocktail.

Reverse transcription-polymerase chain reaction (RT-PCR)

Poly (A)⁺ RNA from each cell line was used for RT-PCR. Each reaction mixture contained 5 μ g total RNA, 50 pmol oligo (dT) primer for 5'-GGCCACGCGTGCGACTAGTAC(T)₁₆-3' and 200 U SuperScriptTM II reverse transcriptase (Invitrogen, Gronigen, Netherlands) in 20 μ l cocktail.

After denature of mRNA at 70°C for 10 min, the reactions were pre-incubated for 2 min at 42°C then incubated at 42°C with reverse transcriptase for 50 min and finally denatured at 70°C for 15 min. Reaction mixture for PCR amplifications contained 1.5 mmol/l MgCl₂ 1 × PCR buffer, 0.2 mmol/l dNTPs, 0.5 U ExTaq polymerase (Takara, Kyoto, Japan) and 0.5 pmol of each forward and reverse primers in 20 µl cocktail. All specific primers used were listed in Table 1. As an internal standard, β -actin was also amplified using the following primers: 5'-TCAGAAGGACTCCTATGTGG-3' and 5'-TCTCTTTGATGTCACGCACG-3' (GenBank, M10277).

Cloning of the myosin light chains from human platelets and U937

The platelet MLC was amplified from cDNA samples by PCR. The nucleotide sequences of the primers were as follows: the forward primer, PL-MLC2-1 5'-CGGAATTAAACAACCACCATGTC-3', which corresponds to the nucleotide number of 1-23 and the reverse primer, Oligo (dT) R 5'-GGCCACGCGTGCAGTAGTAC-3'. The reaction condition was follows: initial denature at 94°C for 30 seconds, 35 cycles of 95°C for 30 seconds, 56°C for 30 seconds, 72°C for 1 minute, and the final extension at 72°C for 7 minutes. PCR-amplified products were gel-purified and subcloned using pGEM-T Easy vector (Promega, Madison, WI, USA) for sequencing.

The terminal amplification of cDNA ends (RACE) method was done to isolate terminal cDNA fragments of MLCs, using a 3'-RACE PCR method as previously described (Watanabe *et al.*, 2001). PCR products were gel-purified and subcloned into the pGEM-T Easy vector and clones were selected and sequenced in both directions.

In the case of U937, the forward primer 5'-ACTTCTTCGCACCAGGAAGC-3' is quoted from the 5' non-coding lesion of smooth muscle MLC-2 (gizzard LC20-A). The procedure of sequencing is the same as that of human platelets.

Sequence analysis

DNA sequences were determined by an ABI PRISMTM377 DNA sequencer (Perkin Elmer Corp., Foster City, CA, USA) and the Dynamic ET Terminator Cycle Sequencing Kit (Amewsham Biosciences, USA). The GENETYX-MAC ver.10 program (Software Development, Co., Ltd., Tokyo, Japan) was used for nucleotide sequence analysis.

Results

Amino acid sequences of predicted myosin light chain (MLC)-2B and 2C

Based on the sequence of myosin light chain MLC-2A (Watanabe *et al.*, 2001) from Meg-01 (Ogura *et al.*, 1985), we amplified cDNA from platelets by RT-PCR using primers, which cover 3' end (3'-GAGAGTTTAAATGGCACAGATCCTG-5' and 5' sequence (5'-CGGTAAACAACCACCATGTC-3') of MLC-2A. We sub-cloned PCR products and determined nucleotide sequence of each clone. In addition to MLC-2A, we obtained two novel clones, which we named as MLC-2B from platelets and MLC-2C from U-937 cells.

Figure 1 shows the nucleotide sequences and derived amino acid sequences for the platelet MLC-2 cDNA (MLC-2B) and U937 MLC-2 cDNA (MLC-2C). The identical nucleotide sequence

A

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1   CGGAATTAACAACCAACCCATGTCGAGCAAAAGAACAAGACCAAGACCAAGAAGCGCCCT   60
      M S S K R T K T K T K K R P
15  CAGCGTGCAACATCCAATGTGTTGCTATGTTTGACAGTCACACAGATTTCAGGAGTTCAAA   120
      Q R A T S N V F A M F D Q S Q I Q E F K
35  GAGGCCTTCAACATGATTGATCAGAACAGAGATGGTTTCATCGACAAGGAAGATTTGCAT   180
      E A F N M I D Q N R D G F I D K E D L H
55  GATATGCTTGCTTCATTGGGAAAGAATCCAACCTGATGAGTATCTAGATGCCATGATGAAT   240
      D M L A S L G K N P T D E Y L D A M M N
75  GAGGCTCCAGGCCCATCAATTTACCCTGTTCTCACCCTGTTTGGTGAGAGTTTAAAT   300
      E A P G P I N F T M F L T M F G E K L N
95  GGCACAGATCCTGAAGATGTCTACAGAAATGCCTTTGCTTGCTTTGATGAAGAAGCAACT   360
      G T D P E D V I R N A F A C F D E E A T
115 GGCACCATACAGGAAGATTACTTGAGAGAGCTGCTGACAACCATGGGGGATCGGTTTACA   420
      G T I Q E D Y L R E L L T T M G D R F T
135 GATGAGGAAGTGGATGAGCTGTACAGAGAAGCACCTATTGATAAAAGGGGAATTTCAAT   480
      D E E V D E L Y R E A P I D K K A M M N
155 TACATCGAGTTCACACGCATCCTGAAACATGGAGCCAAAGACAAAGATGACTGAAATAAC   540
      Y I E F T R I L K H G A K D K D D ***
      TTCAAATTCAGCCAAACGTTTCCTTGTGGCACTTTGGGTATTCTGAGATTTTCTCTTGC   600
      ATGCCCTTAGCTTTACAGCTTTTTCGATTTCTGTTGATTTTATTCTCAGCCATTTTGGGC   660
      ATATGATCTTTATAATACAGACTGGAACCGGACTTTCTATTAATATCATTTTCAGAAATA   720
      AAAAAAGGGTAATTTAAACCTACCAAGCCCTTCTCCCCCAATAAAGTGTGTTCTATACAGAG   780
      TCAATATATTTTTTCAGAGAAAGTTATTCGCTCGATTTTCTTCTGAATCATAAATTAAGCTT   840
      TATGATAAAATACTTAAAAAAAAAAAAAAAAAAAAAAAAA

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B

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1   TTCTTCGCACCAAGGAAGCCCCACCCACCAAGCAAGATGTCCAGCAAGCGGGCCAAA   60
      M S S K R A K
8   GCCAAGACCACCAAGAAGCGGCCACAGCGGGCCACATCCAATGTCTTCGCAATGTTTGAC   120
      A K T T K K R P Q R A T S N V F A M F D
28  GCCAAGCAGATCCAGGAGTTTAAGGAGGCTTTCAACATGATTGACCAGAACCCTGATGGC   180
      Q S Q I Q E F K E A F N M I D Q N R D G
48  TTCATTGACAAGGAGGACCTGCACGACATGCTGGCCTCGCTGGGGAAGAACCCACAGAC   240
      F I D K E D L H D M L A S L G K N P T D
68  GAATACCTGGAGGGCATGATGAGCGAGGCCCGGGGCCATCAACTTCACCATGTTCTCTC   300
      E Y L E G M M S E A P G P I N F T M F L
88  ACCATGTTTGGGAGAAGCTGAACGGCACGGACCCCGAGGATGTGATTTCGCAACGCCTTT   360
      T M F G E K L N G T D P E D V I R N A F
108 GCCTGCTTCGACGAGGAAGCCTCAGGTTTCATCCATGAGGACCACCTCCGGGAGCTGCTC   420
      A C F D E E A S G F I H E D H L R E L L
128 ACCACCATGGGTGACCGCTTCACAGATGAGGAAGTGGACGAGATGTACCGGAGGCACCC   480
      T T M G D R F T D E E V D E M Y R E A P
148 ATTGATAAGAAAGGCAACTTCAACTACGTGGAGTTCACCCGCATCCTCAAACATGGCGCC   540
      I D G K K G N F N Y V E F T R I L K H G A
168 AAGGATAAAGCAGCTAGGCCACCCAGGAGGAAGAGGCCCTGAGTCTGGGATCAGACA   600
      K D K D D ***
      CCCCTTCACGTGTATCCCCACACAAATGCAAGCTCACCAAGGTCCCCTCTCAGTCCCCTT   660
      CCCTACACCCCTGACCGGCCACTGCCGACACCCACCCAGAGCACGCCACCCGCCATGGGA   720
      GTGTGCTCAGGAGTCCGCGGCAGCGTGGACATCTGTCCAGAGGGGGCAGAATCTCCAAAT   780
      AGAGGACTGAGCACTGAAAAAAAAAAAAAAAAAAAAA

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Fig. 1. The nucleotide and deduced amino acid sequences of the full length cDNA for the platelet MLC-2 cDNA (A) and the U937 MLC-2 cDNA (B). The deduced amino acid sequence of MLC-2 is shown in single-letter code under the nucleotide sequence. Nucleotides are numbered on the right of each line, and numbers on the left are those of amino acid residues starting from methionine-1. The termination codon is represented by asterisks and a putative additional poly (A) signal AATAA(G)A is underlined.

to U937 was obtained from HEL cells (data not shown).

Figure 2 shows the deduced amino acid sequences for the MLC-2B and MLC-2C in comparison with MLC-2A, human vascular smooth muscle LC-20 (Kumar *et al.*, 1989), chicken

Fig. 2. Comparison of the MLC-2A protein sequences with MLC-2B, MLC-2C and other published MLCs sequences. The amino acid sequences of MLC-2A and identical residues in other proteins are shaded. Gaps were introduced to maintain the alignment. Asterisks indicate published phosphorylation sites. human sm; human vascular smooth muscle LC-20, gizzard L20-A; chicken gizzard smooth muscle L20-A, gizzard L20-B; chicken gizzard smooth muscle L20-B.

Fig. 3. Similarities of three isoforms on nucleotide and amino acid. Denominator is the similarity of amino acid and numerator is that of nucleotide.

gizzard smooth muscle L20-A (Messer and Kendrick-Jones, 1988; Zavodny *et al.*, 1988), chicken gizzard smooth muscle L20-B (Inoue *et al.*, 1989). Amino acid sequence of MLC-2C was, of interest, apparently almost the same as the sequence of MLC-2 from chicken gizzard smooth muscle L20-A (one amino acid's difference) and human vascular smooth muscle LC-20 (two amino acids' difference). All three protein kinase C (PKC) phosphorylation residues (Ser-1, Ser-2, Thr-9) and both myosin light chain kinase (MLCK) phosphorylation residues (Thr-18, Ser-19) are conserved in these three isoforms.

Figure 3 shows the similarity of amino acid and nucleotide among these three MLC-2 isoforms. MLC-2B differed in four and ten amino acid when compared with MLC-2A and MLC-2C, which revealed the similarity of 97.7% and 93.0% respectively. MLC-2B differed in 25 and 109 nucleotides when compared with MLC-2A and MLC-2C, which revealed the similarity of 95.2%

Fig. 4. Structure of three MLC-2 genes. ▽; initiation codon, ▼; stop codon

and 78.9%, respectively.

Structure of MLC-2 genes (Fig. 4)

Analysis by computer search (NCBI) revealed that both MLC-2A and MLC-2B consisted of three exons, which were situated on gene loci 18p1.3. Analysis of the gene structure indicated that MLC-2A and MLC-2B utilized different exons. MLC-2C also consisted of three exons, which was situated on gene loci 20p12.

Expression of MLCs mRNA in the human hematopoietic cells (Fig. 5)

To examine the expression of MLC genes RT-PCR analysis was done using total RNA isolated from following blood cells; human acute lymphocytic leukemia (Reh), pre-B cell leukemia (NALM-6), B lymphoid leukemia (Daudi and Raji), plasma cell leukemia (U266, RPMI8226 and NC37), acute T cell leukemia (Jurkat and HUT78), myeloid leukemia (HL-60), monocytic leukemia (THP-1, A-THP-1 and U937), erythroid leukemia (K562 and HEL), megakaryoblastic leukemia (Meg-01 and CMK) and cord blood cells. Primer pairs, which discriminated MLC-2A, -2B and -2C were used (Table 1). The MLC-2A and MLC-2B mRNA were expressed in all of the leukemic cell lines examined and their expression level were almost the same. On the other hand, MLC-2C mRNA was expressed in monocytic cell lines (U937 and A-THP-1) and erythroid leukemia cell lines (K562 and HEL) and its expression was relatively low as compared with that of cord blood cells.

We employed the well-established *in vitro* differentiation system utilizing human myeloid leukemia cell line, HL-60. TPA can efficiently induce terminal differentiation of HL-60 cells into a monocyte/macrophage phenotype, while dimethyl sulphoxide (DMSO) can induce into a

Fig. 5. Expression of MLCs mRNA in the human hematopoietic cells. RT-PCR analysis was done using total RNA isolated from seventeen kinds of human hematopoietic cell lines and cord blood cells. β -actin (500 bp) was used as an internal standard. β -actin mRNA was readily detectable in all the cell lines used. M, molecular-weight markers ($\phi \times 174$ -Hae III: Nippon Gene).

Table 1. The Primer Sequences of RT-PCT for MLC-2A, -2B and -2C

Target cDNA	Primer Sequence (5'–3')	Anneling (°C)	Product sizes (bp)
MLC-2A	5' CAAAGACCAAGACCACCAAGAAG 3' CAGGATCTGTGCCATTAACTTC	60	282
MLC-2A	5' ACCACCATGTAGAGCAAAAAGG 3' CAGGATCTGTGCCATTAACTTC	61	304
MLC-2B	5' CAAAGACCAAGACCAAGAAGCGC 3' CAGGATCTGTGCCATTAACTTC	60	279
MLC-2B	5' AACCACCATGTGCGAGCAAAAAGAA 3' CAGGATCTGTGCCATTAACTTC	61	302
MLC-2C	5' ATGTCCCAGCAA(A/G)CG(G/T)GCCAAA 3' GCCTG(A/G)CTCATCAT(C/G)CCCTC	63	230

granulocyte phenotype (Trayner *et al.*, 1998). We next examined induction of MLC-2C during differentiation of HL-60 into monocyte/macrophage cell lineage. RT-PCR analysis showed that neither MLC-2A nor MLC-2B increased in levels in response to TPA treatment in HL-60 cells. On the other hand MLC-2C expression was apparently induced at the point of 12 hours and reached its peak at 48 hours after TPA treatment (Fig. 6). The dose dependency of TPA on MLC-2C expression was also observed (data not shown). MLC-2C was not induced in DMSO-treated HL-60 cells (Fig. 7). Furthermore neither TPA-treated Meg-01 nor CMK showed MLC-2C expression in our condition (Fig. 7).

Fig. 6. RT-PCR analysis of three isoforms in response to TPA treatment in HL-60 cells. mRNA was extracted at the incubation time of 0h, 6h, 12h, 18h, 24h, 48h, and 72h. β -actin (500 bp) was used as an internal standard. M, molecular-weight markers ($\phi \times 174$ -Hae III: Nippon Gene).

Fig. 7. RT-PCR analysis of HL-60 treated with DMSO or TPA in comparison with CMK and Meg-01. M, molecular-weight markers ($\phi \times 174$ -Hae III: Nippon Gene). lane 1, HL60, untreated; lane 2, HL60, 1.3%DMSO, 3×10^4 cells, 4 days; lane 3, HL60, 1.3%DMSO, 5×10^4 cells, 4 days; lane 4, HL60, 1.5%DMSO, 3×10^4 cells, 4 days; lane 5, HL60, 20 nM TPA, 3×10^4 cells, 3 days; lane 6, HL60, 20 nM TPA, 5×10^4 cells, 3 days; lane 7, HL60, 50 nM TPA, 3×10^4 cells, 3 days; lane 8, HL60, 50 nM TPA, 5×10^4 cells, 3 days; lane 9, CMK, untreated; lane 10, CMK, 50 nM TPA, 3×10^4 cells, 3 days; lane 11, CMK, 50 nM TPA, 5×10^4 cells, 3 days; lane 12, Meg-01, untreated; lane 13, CMK, 50 nM TPA, 3×10^4 cells, 3 days; lane 14, CMK, 50 nM TPA, 5×10^4 cells, 3 days.

Discussion

Recent structure-function analysis of smooth muscle MLC-2 revealed that the C-terminal portion of MLC-2 is important for regulation by phosphorylation and critical for the binding to the heavy chain (Trybus *et al.*, 1993). It is postulated that phosphorylation at the N-terminal region of the MLC-2 causes the conformational change of MLC-2 and this change in the conformation is transmitted to the heavy chain via the C-terminal portion of MLC-2 (Trybus, *et al.*, 1993; Kamisoyama *et al.*, 1994; Ikebe *et al.*, 1994).

We previously described on sequence analysis of cloned cDNAs of MLC-2, named as MLC-2A and 17 kDa essential myosin light chain from the human megakaryoblastic leukemia cell line, Meg-01 (Watanabe *et al.*, 2001). This was apparently the first documentation of cloning of full-length cDNA of MLC-2 from human nonmuscle cells. We herein reported two another isoforms of MLC-2 (named as MLC-2B and MLC-2C) from human hematopoietic cells (Figs. 1

and 2).

Phosphorylation of MLC-2 initiates the contraction of vertebrate smooth muscle (Ikebe *et al.*, 1984). In vitro, high concentrations of MLCK phosphorylate threonine (Thr)-18 in addition to serine (Ser)-19 and markedly increase actin-activated ATPase activity in both gizzard smooth muscle (Ikebe *et al.*, 1986) and platelets (Ikebe, 1989; Higashihara *et al.*, 1991). MLC-2 is also a substrate for protein kinase C (PKC) and p34^{cdc2} kinase which phosphorylate three residues on the N-terminus of the MLC-2, Ser-1, Ser-2, and Thr-9 (Ikebe, 1986; Ikebe, 1987). All three protein kinase C (PKC) phosphorylation residues (Ser-1, Ser-2, Thr-9) and both myosin light chain kinase (MLCK) phosphorylation residues (Thr-18, Ser-19) are conserved in these three isoforms (Figs. 1 and 2). Moreover, in three isoforms, sequence ¹³Arg to ¹⁶Arg which is important for acto-ATPase activity in gizzard smooth muscle (Ikebe *et al.*, 1994) was conserved. The nucleotide sequence of MLC-2C was almost the same as that of chicken gizzard smooth muscle myosin L20-A (one amino acid's difference) and that of human vascular smooth muscle LC-20 (two amino acids' difference) (Fig. 2). Thus we propose to refer non-muscle MLC-2C as smooth muscle type MLC-2. Computed search revealed that these three isoforms originated in different genes. MLC-2A gene is located in the same chromosome of 18p13.3 as MLC-2B gene. In contrast, MLC-2C gene is located in 20p12 (Fig. 4).

MLC-2A and MLC-2B were constitutively conserved in all hematopoietic cells determined. MLC-2C was observed only in monocytic cell lines and erythroid cell lines (Fig. 5). To be of interest, HL-60 differentiated into monocyte/macrophage cell lineage by TPA treatment expressed MLC-2C (smooth-muscle type), although its amount was low in compared with that of cord blood cells (Fig. 6). MLC-2C may play a crucial role in, at least, monocyte/macrophage cell lineage. The functional role of MLC-2C in erythroid leukemia cells (K562 and HEL) also need to be determined. Neither CMK nor Meg-01 treated with TPA, which is thought to be differentiated into mature megakaryocytic cell lineage, showed MLC-2C expression (Fig. 7).

Although we cannot neglect the existence of other novel MLC-2 isoforms, these three isoforms might be expressed in a different ratio, having the different cellular functions in human hematopoietic cells.

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