Journal of Cellular Biochemistry 94:763–773 (2005)

Developmental Regulation and Cellular Distribution of Human Cytosolic Malate Dehydrogenase (MDH1)

Agnes Shuk-Yee Lo,[1] Choong-Tsek Liew,[2] Sai-Ming Ngai,[1] Stephen Kwok-Wing Tsui,[1] Kwok-Pui Fung,[1] Cheuk-Yu Lee,[1] and Mary Miu-Yee Waye[1]*

[1]Department of Biochemistry, Croucher Laboratory for Human Genomics and The Hong Kong Bioinformatics Center, The Chinese University of Hong Kong, Hong Kong SAR, China
[2]Department of Anatomical and Cellular Pathology, The Prince of Wales Hospital, Hong Kong SAR, China

Abstract Human cyotsolic malate dehydrogenase (MDH1) is important in transporting NADH equivalents across the mitochondrial membrane, controlling tricarboxylic acid (TCA) cycle pool size and providing contractile function. Cellular localization studies indicate that MDH1 mRNA expression has a strong tissue-specific distribution, being expressed primarily in cardiac and skeletal muscle and in the brain, at intermediate levels in the spleen, kidney, intestine, liver, and testes and at low levels in lung and bone marrow. The observed MDH1 localizations reflect the role of NADH in the support of a variety of functions in different organs. These functions are primarily related to aerobic energy production for muscle contraction, neuronal signal transmission, absorption/resorption functions, collagen-supporting functions, phagocytosis of dead cells, and processes related to gas exchange and cell division. During neonatal development, MDH1 is expressed in human embryonic heart as early as the 3rd month and then is over-expressed from the 5th month until the birth. The expression of MDH1 is maintained in the adult heart but is not present in levels as high as in the fetus. Finally, over-expression of MDH1 is found in left ventricular cardiac muscle of dilated cardiomyopathy (DCM) patients when contrasted to the diseased non-DCM and normal heart muscle by in situ hybridization and Western blot. These observations are compatible with the activation of glucose oxidation in relatively hypoxic environments of fetal and hypertrophied myocardium. J. Cell. Biochem. 94: 763–773, 2005. © 2004 Wiley-Liss, Inc.

Key words: heart cDNA; heart energy metabolism; human cytosolic malate dehydrogenase (MDH1); developmental expression; in situ hybridization; dilated cardiomyopathy (DCM)

NAD^+-dependent malate dehydrogenase (MDH) (L-malate: NAD^+ oxidoreductase, EC 1.1.1.37) catalyses the reversible conversion of L-malate to oxaloacetate accompanied by the exchange of reducing equivalents across the mitochondrial membrane [Webb et al., 1973]. It plays a crucial role both in the malate-aspartate shuttle and the citric acid cycle in all aerobic tissues of mammals [Grant et al., 1987]. In eukaryotic cells, two MDH isozymes are encoded by nuclear genes and are synthesized in the cytoplasm. Cytosolic MDH (MDH1) remains in the cytosol after synthesis whereas mitochondrial MDH (MDH2) is translocated into the mitochondrial matrix facilitated by an amino-terminal polypeptide signal [Aziz et al., 1981; Grant et al., 1987; Hartmann et al., 1993].

Despite a high degree of 3-dimensional structure conservation, the amino acid sequence

Abbreviations used: CPT-1, carnitine palmitoyltansferase 1; MDH1, cytosolic malate dehydrogenase; DCM, dilated cardiomyopathy; MDH2, mitochondrial malate dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; TCA, tricarboxylic acid.

Sequence data reported in this article have been deposited in GenBank/EMBL with the accession number U20352.

Grant sponsor: Research Grants Council, Hong Kong; Grant sponsor: Innovation and Technology Commission, Government of Hong Kong SAR, China; Grant sponsor: Croucher Foundation; Grant numbers: CUHK 205/96M, CUHK 4305/99M, AF/47/98, AF/9/97; Grant sponsor: Strategic Research Area Grant.

*Correspondence to: Prof. Mary Miu-Yee Waye, Department of Biochemistry, Room 608, Mong Man Wai Building, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong. E-mail: mary-waye@cuhk.edu.hk.

Received 4 June 2004; Accepted 7 September 2004

DOI 10.1002/jcb.20343

© 2004 Wiley-Liss, Inc.

homology between the two eukaryotic isozymes ranges from 20% to 25% across species. By contrast, mouse MDH1 shares 56% identity with *E. coli* MDH and mouse MDH2 has 52% homology with *T. flavus* MDH [Nishiyama et al., 1986; Joh et al., 1987a,b; Hall et al., 1991]. MDH1 mRNA (1.4 kb) and MDH2 mRNA (1.3 kb) are expressed in a similar pattern and degree in a variety of tissues. Northern blot analysis indicates that mRNAs of the mouse MDHs and human MDH1 are widely distributed, particularly in tissues with high metabolic demand for NADH produced by the tricarboxylic acid (TCA) cycle: high levels in the heart, skeletal muscle, and brain, intermediate levels in smooth muscle and kidney, and low levels in the liver [Joh et al., 1987a; Tanaka et al., 1996]; however, the cellular localization of MDHs has not yet been characterized.

Exercise training has been shown to increase the levels of glycolytic and oxidative enzymes in heart and skeletal muscle [Takekura and Yoshioka, 1990; Stuewe et al., 2000]. With muscle fiber hypertrophy, there is a significant increase in the oxidative enzymes succinate dehydrogenase (SDH) and MDH induced by sprint training and a further increase following endurance training in rat leg muscle [Takekura and Yoshioka, 1990]. This illustrates enzymatic adaptations in the energy-generating machinery of the cell in order to meet the energy needs of increased contractile power. The metabolic environment of fetal heart primarily relies on carbohydrates to increase oxygen-efficient production of ATP in a relatively hypoxic environment. Increased reliance on carbohydrate oxidation for energy needs parallels an increased expression of MDH as part of the TCA cycle. After birth, the heart switches to β-oxidation of fatty acids with a large ATP production capacity that matches the high oxygen consumption needs of the adult heart [Sack et al., 1996; Barger and Kelly, 1999]. In cases of human cardiac hypertrophy and heart failure, it has been suggested that the heart reverts to a fetal metabolic pattern, utilizing glucose and lactate as energy sources with the reactivation of a variety of developmental transcription factors, embryonic genes and contractile proteins [Chien et al., 1991; Razeghi et al., 2001]. This energy metabolic transition allows hypertrophied ventricles to satisfy a high-energy demand in the low oxygen conditions of a diseased heart at the cost of diminished contractile reserve. These observations suggest the hypothesis that hypertrophied heart might reflect a fetal expression pattern with increased MDH1 levels.

Our research attempts to identify genes associated with heart development and heart diseases in conjunction with the large-scale sequencing of a human heart cDNA library. Here, we show that MDH1 expression parallels tissue carbohydrate energy expenditure. High levels of MDH1 are present mainly in the striations of heart and skeletal muscle. Conditions of hypoxia that increase dependency on carbohydrate energy sources induce MDH1 expression to even higher level in fetal and dilated hearts.

MATERIALS AND METHODS

MDH1 Clones Isolation and Sequence Organization

Large-scale partial sequencing of an adult human heart cDNA library (Clontech, Palo Alto, CA) was performed as described by Liew et al. [1994]. Sequence comparisons against the GenBank/EMBL nucleotide and protein databases were performed using the BLAST server. Four clones which were found to have DNA sequences resembling pig or mouse MDH1 provided information for the design of a 5′ cloning primer to screen the cDNA libraries for the complete coding region of human species of MDH1. The complete sequence of MDH1 cDNA was obtained via primer walking using the AutoRead Sequencing Kit and an automated A.L.F. sequencer (Pharmacia, Piscataway, NJ). The full-length cDNA sequence of MDH1 was submitted to GenBank with accession no. U20352. The 5′ untranslated sequence was obtained by the 5′ RACE method using Marathon-Ready cDNA (Clontech). Exon/intron junction sequences were gained through the use of genomic DNA extracted from human blood. The length of each intron was determined by PCR using primers located in exon sequences. A cDNA probe spanning exons 4 and 5, which includes the conserved coenzyme-binding domain amongst mammalian MDH1 (no homology with that of MDH2), was labeled with DIG according to the manufacturer's instructions (Boehringer Mannheim, Mannheim, Germany) (forward: 5′-GTT ATT GTT GTG GGT AAT CCA G-3′ and Reverse: 5′-CGT GAC AAA TTC TCC CTT GAG C-3′). The yield of the DIG-labeled probe was about 10 ng/ml.

Sample Items and History of Patients

The following tissues were used to investigate the expression profile of MDH1 in embryonic development, tissue distribution, and heart disease state by in situ hybridization. (I) Human fetal heart tissues from 3M (month), 4M, 5M, 6M, and 7M from three individuals were used to assess the expression regulation of MDH1 during embryonic development. (II) Samples of multiple human and/or rat organ types were used to study the tissue distribution including heart, brain, lung, kidney, spleen, liver, bone marrow, skeletal muscle (from rat only), sexual organs (from rat only), and intestine (from rat only). The tissues were obtained from three individuals or from three different rats. Owing to the fact that the autopsy samples of human skeletal muscle and sexual organs were not available and gastro-intestinal tract samples were autolysed several days after death, the rat samples of corresponding organs were used. (III) Lastly, left ventricular myocardial muscle of two dilated cardiomyopathy (DCM) surgical samples (the same samples as for the Western blot, see below) and three normal human heart tissues which are not suitable for heart transplantation were obtained to examine the expression level of MDH1 in the disease state. The tissue samples were stored in 10% buffered formalin for tissue section processing.

For the Western blot, the left ventricular myocardial muscle samples from DCM patients were excised, and non-DCM heart disease biopsies and normal heart tissue at the same location were obtained from the Department of Surgery of Prince of Wales Hospital, Shatin, Hong Kong. The patients' histories were recorded in details as follows: (sex/age/diagnosis/type of operation) (1) Diseased 1 (D1): M/62/Dilated Cardiomyopathy, DCM/left ventricular reduction and mitral valve replacement; (2) Diseased 2 (D2): M/48/Dilated Cardiomyopathy, DCM, severe aortal regurgitation and dilated left ventricle/double valve replacement and partial left ventriclectomy; (3) Non-DCM 1 (ND1): F/60/chronic rheumatic heart disease/aortic regurgitation and aortic valve replacement; (4) Non-DCM 2 (ND2): F/37/mitral regurgitation/mitral valve replacement. (5) The normal tissue (N) is obtained from 68-year-old male. The excised tissues were snapped frozen immediately and stored at $-80°C$ until protein extraction.

In Situ Hybridization and Grading of Expression

The tissue slides were dewaxed, rehydrated, and finally washed in a 0.05M TBS pH7.6 (Tris-buffered saline solution, 0.05M Tris-HCl pH 7.6, 0.15M NaCl). They were treated with several drops of pepsin-HCl for 10 min at 37°C and then washed in TBS. A DIG-labeled cDNA MDH1 probe with concentration of about 100 pg/ml or 1:20 dilution was applied to the sections. They were denatured at 95°C for 10 min, then chilled on ice immediately and finally incubated at 42°C overnight for hybridization. The slides were then washed with TBS three times, and incubated in a prewarmed stringent wash solution (1.5 mM sodium citrate, 15 mM NaCl, 0.1% Triton X-100, 0.05% BSA, and 2 mM $MgCl_2$) at 42°C for 30 min. The tissues were washed with TBS. Afterwards, the tissues were blocked with 5% goat serum for 10 min, probed with anti-DIG alkaline phosphatase (1:300) for 30 min and incubated with substrate BCIP/NBT for up to 2 h. They were counterstained with 0.1% nuclear red, dehydrated, and fixed with mounting medium for preservation. A single batch of probe was used to generate all the data presented and a uniform time and temperature of incubation for the development of the signal in the alkaline phosphates substrate was used. During hybridization, the unlabeled probe or in the absence of probe was used to serve as a negative control of staining.

The intensities of the MDH1 mRNA expression on tissues as determined by DIG detection system were grouped into five grades with 0, I, II, III, and IV considered to represent no, low, intermediate, high, and over-expression, respectively.

Western Blot Analysis

The frozen tissues were suspended into lysis buffer (8M urea, 0.1M sodium phosphate, 0.01M Tris/HCl, 10 mM DTT at pH 8.0) and then homogenized. The supernatant solution, containing total cardiac protein, was collected, normalized using a Bio-Rad protein assay kit. The samples were loaded onto a 12% SDS–PAGE gel at 150 μg per lane. Anti-MDH1 polyclonal antibodies were raised against an acetylated peptide corresponding to the 5′-end of MDH1 sequence (Ac-CTGAAGQIAY). The peptide conjugate was purified with a G-50 Sephadex desalting column (Pharmacia) and injected

into rabbit with Freund's adjuvant. SDS gels were then placed into transfer buffer (25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3) for 20 min and transferred onto a cellulose paper overnight. The filter was blocked with 5% non-fat milk powder in TBST (0.05% Tween-20) for 1 h, probed with primary antibody anti-MDH1 (1:300) at 4°C overnight and incubated with secondary antibody HRP labeled goat anti-rabit (1:3,000) for 1 h. The signal was detected by enhanced chemiluminescence (ECL) detection reagents (Amersham, Life Science, Arlington Heights, IL). Intensity of the target bands on the nitrocellulose membrane was measured by densitometer (Bio-Rad, Hercules, CA Model GS-670).

RESULTS

Isolation of *MDH1* Gene and Sequence Organization

A MDH1 cDNA clone was isolated during our human heart cDNA sequencing project and its sequence was revised using high fidelity *PWO* polymerase (Boehringer Mannheim) with GenBank accession no. U20352. It is 1,212 bp in length with predicted open reading frame (ORF) encoding 334 amino acids with 207 bp in the 3′-untranslated region and 62 bp in the 5′-untranslated region as detected using the 5′-RACE method. The *MDH1* gene is located in chromosome 2 (with the cytogenic location of 2p13.3) [Lo et al., 1999] and is encoded by seven exons. The conservation of the eight intron positions of cytosolic and mitochondrial MDHs suggests that a common ancestral gene for MDH1 and MDH2 was interrupted by introns before the two genes diverged [Grant et al., 1987; Joh et al., 1987a,b]. All exon–intron boundaries were found to follow the GT-AG rule of splice donor/acceptor sites (data not shown).

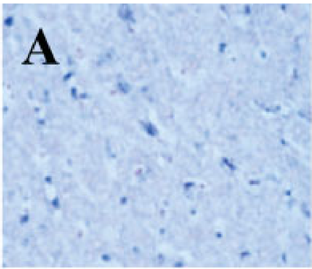
The amino acid sequence of MDH1 was highly homologous to those of mouse and pig MDH1 with identities of 96.1% and 95.5%, respectively. In contrast, MDH1 shares only 17.9% identity of amino acids with MDH2. Additionally, the homology between MDH1 and *Thermus flavus* MDH (55.1%) is much higher than that with other bacterial species (*E. coli*: 27.8%; *H. marismortui*: 21.1%). Graphic analysis on a Silicon Graphics workstation revealed that there are five unique human amino acid residues (a.a. 75, 76, 176, 224, and 326) located primarily on the surface of the protein; however, these residues were not found in the conserved human coenzyme and substrate binding sites. For in situ hybridization, exon 4–5 cDNA was designed as a probe based on the highly conserved coenzyme-binding domain amongst mammalian MDH1 [Joh et al., 1987a,b]. This domain does not share particular homology with that of MDH2 after blast sequence search. Subsequently, MDH1 cDNA was also cloned into expression vector pAED4 and expressed in *E. coli* with molecular weight of 37 kDa (data not shown). Protein expression analysis of MDH1 demonstrated that the expressed enzyme was functionally active in the presence of at least 0.125 mM NADH in PBS, showing an average increase of 53.5-fold in tetrazolium salt staining intensity on native gel when compared with uninduced protein or PBS controls (data not shown).

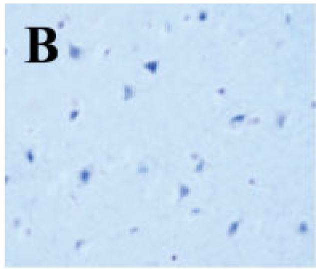
Cellular Distribution of MDH1 mRNA in Tissues

In situ hybridization is used for measuring the intensity of expression and to delineate the specific localization of a particular gene. MDH1 mRNA was expressed at a high level in heart and skeletal muscle, at a relatively high level in the brain and spleen, at an intermediate level in the kidney, intestine, liver, and testes, and at a low level in the lungs and bone marrow. In general, the distribution of MDH1 correlates with locations of high cellular energy metabolism such as myocardial muscle, skeletal muscle, and intestinal smooth muscle. Especially strong staining was observed at muscle striations (Fig. 1A). ATPase energy production is required in neuronal signal transmission in astrocytes in the brain (Fig. 1B) using NADH and O_2 for metabolism. ATPase is also involved

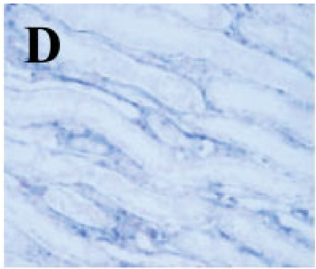
Fig. 1. Cellular localization of cytosolic malate dehydrogenase (MDH1) mRNA by in situ hybridization. The tissue distribution is indicated in brackets under the organ name. **A**: Skeletal muscle (striations); (**B**) brain (astrocytes); (**C**) spleen (red pulp); (**D**) kidney (medullary collecting tubules); (**E**) kidney (proximal convoluted tubules); (**F**) intestine (the epithelial mucosal gland, smooth muscularis mucosae, and submucosae); (**G**) liver (the sinusoid including Kupffer cells); (**H**) testes (the seminiferous tubules); (**I**) lung (alveolar walls); and (**J**) bone marrow (erythroblastic islands). In situ hybridization indicated the MDH1 mRNA expression in blue signal using DIG-labeled probe with sections counterstained with 0.1% nuclear red. The histology of the corresponding tissues was visualized by hematoxylin and eosin (H&E) staining in lower panel. (400× magnification).

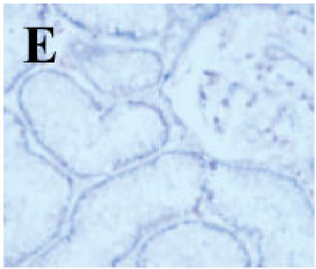
DIG
H&E

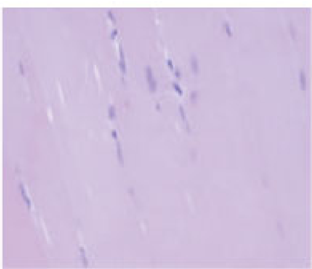

A

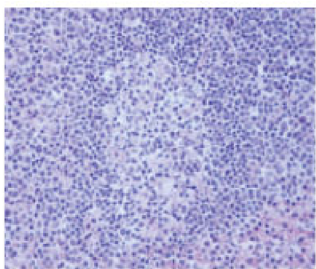

B

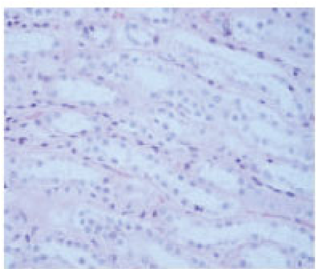
C

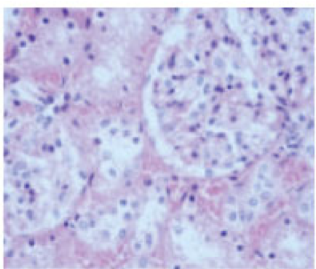

D


E

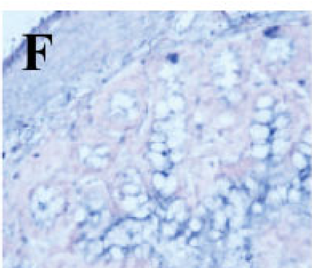


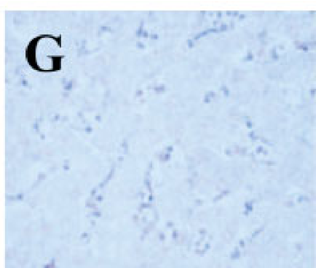


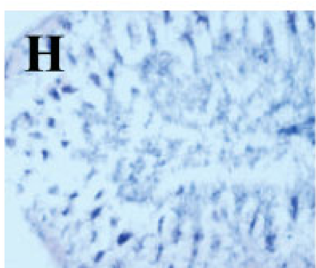


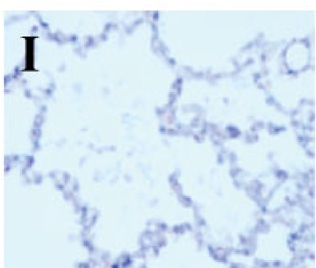


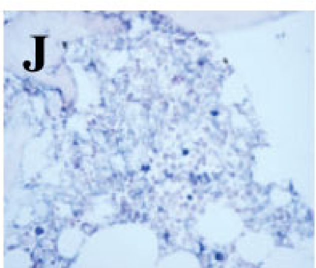
DIG
H&E

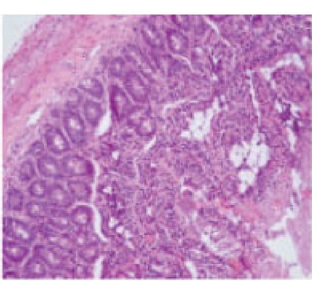

F

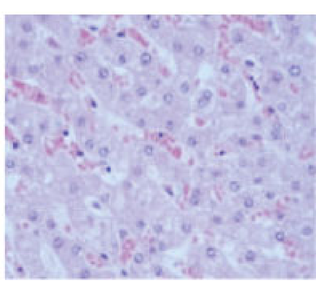

G

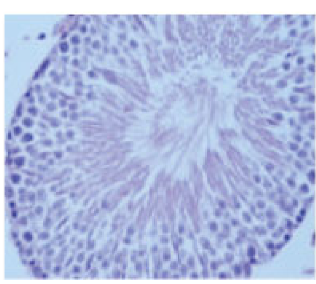

H

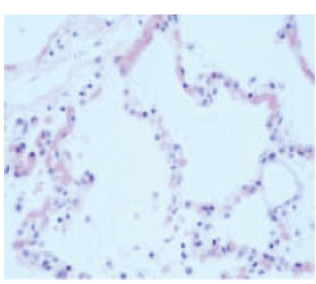

I

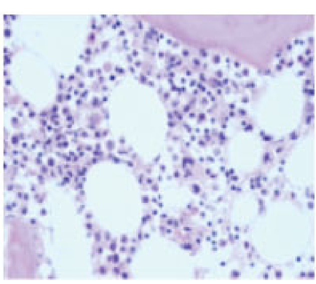

J











in nutrients and fluid absorption in epithelial cells located at intestinal mucosal glands (Fig. 1F) and the proximal convoluted tubules of the kidney (Fig. 1E). MDH1 is localized in the collagen structures of the basement membrane (Fig. 1D) that play a role in maintaining the form of the medullary collecting tubules in the kidney. Another essential function is phagocytosis of dead cells in the red pulp of the spleen (Fig. 1C), the sinusoid Kupffer cells of the liver (Fig. 1G), and possibly in alveolar macrophages (Fig. 1I). Cell division during spermatogenesis in the seminiferous tubules of the testes (Fig. 1H) and during erythropoiesis in bone marrow may also require high energy levels (Fig. 1J). Lastly, MDH1 is involved in the process of gas exchange in cells of the alveolar wall (Fig. 1I). Table I summarizes the expression intensity and cellular localization of MDH1 mRNA, and the postulated physiological functions of MDH1. Similar patterns of MDH1 mRNA cellular localization were found in tissues from both three human individuals and three rats.

Developmental Regulation of MDH1 mRNA Expression

In situ hybridization mainly detects MDH1 at the striations of myocardial muscle throughout the heart. During human embryonic development, MDH1 was expressed as early as the 3rd month in the heart (Fig. 2A) with a marked increase in expression from the 5th month onwards (Fig. 2B). The expression of MDH1 mRNA in the fetus was higher even than that of normal adult human heart (Fig. 3A), indicating significant over-expression.

Over-Expression of MDH1 in DCM

In situ hybridization of tissues demonstrated that myocardial muscle showed over-expression of MDH1 mRNA in DCM myocardium (Fig. 3B) versus normal heart (Fig. 3A). Hematoxylin and eosin (H&E) staining confirmed that representative DCM heart muscle cells were hypertrophied and their nuclei were enlarged compared to normal. In addition, Western blot indicated a 15-fold increase in MDH1 in left ventricular myocardium from DCM patients (D1 and D2) as compared to the protein extracted from the same location of valve-diseased non-DCM heart biopsies (ND1 and ND2) and of normal heart tissue (N1) (Fig. 3C). The hearts of ND1 and ND2 did not exhibit hypertrophy.

DISCUSSION

Our data indicate that the MDH1 cDNA sequence has higher homology with MDH1 nucleic acid sequences of other species than with those of their corresponding mitochondrial isozymes (MDH2) [Nishiyama et al., 1986; Joh et al., 1987a,b; Hall et al., 1991]. This contrasts the citrate synthases, another enzyme family of the TCA cycle, which displays a very high homology between the two eukaryotic isozymes but has a marked dissimiliarity from the prokaryotic types [Hall et al., 1991]. Distinctive residues of human, porcine, and rat sequences of MDH1 occur primarily on the surface of the protein [Grant et al., 1987].

In situ hybridization shows that MDH1 mRNA is ubiquitously distributed but with a high preponderance in the most energy dependent tissues. The degrees of expression in

TABLE I. Summary of the Cellular Expression of MDH1 mRNA in Different Tissues

Organ location	Cellular localization	Functions
High expression level		
Heart	Muscle striations	Muscle contraction
Skeletal muscle	Muscle striations	Muscle contraction
Brain	Astrocytes	Neuronal signal transmission
Intermediate expression level		
Spleen	Red pulp	Phagocytosis
Kidney	Medullary collecting tubules	Collagen-supporting
	Proximal convoluted tubules	Absorption/resorption
Intestine	Intestinal mucosal glands	Absorption/resorption
Liver	Sinusoid Kupffer cells	Phagocytosis
Testes	Seminiferous tubules	Cell division-spermatogenesis
Low expression level		
Lung	Alveolar macrophages	Phagocytosis
	Alveolar wall	Gas exchange
Bone marrow	Erythroblastic islands	Cell division-erythropoiesis

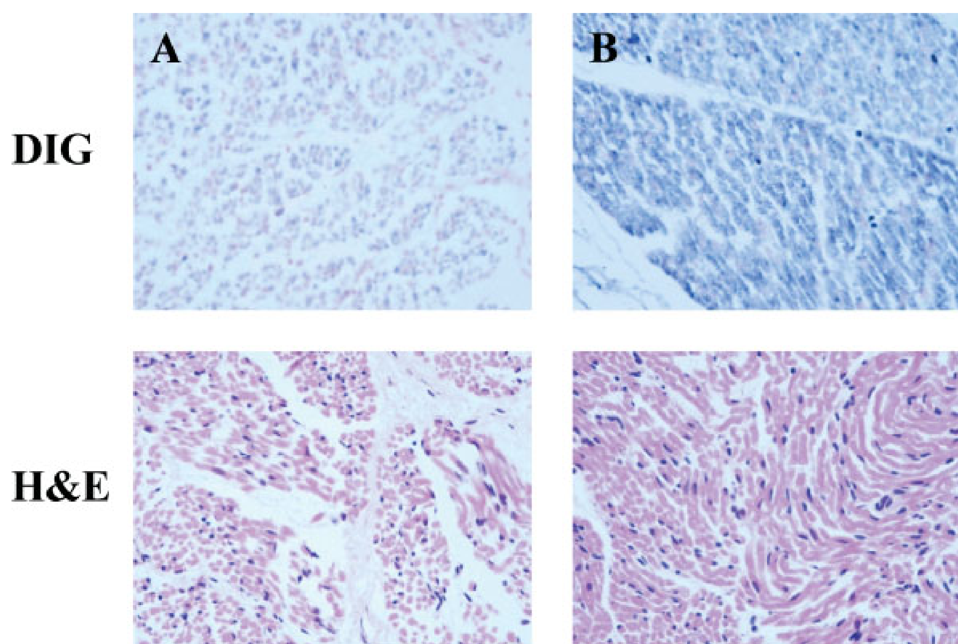


Fig. 2. Developmental regulation of MDH1 in human fetal heart. **A**: The 3rd month human fetal heart and (**B**) the 5th month human fetal heart. Methods as in Figure 1. Similar signals were observed in the tissue samples from three different individuals.

tissues observed here are consistent with previous Northern blot studies on animals [Joh et al., 1987a]. High MDH1 expression is seen in high energy metabolic rate tissues in heart, skeletal muscle, and brain. Its expression is observed in other tissues with middle-high metabolic rates such as kidney, liver, and in the submucosal smooth muscle layers of bowel (Table I). An intravenous perfusion of 125I-labeled rat MDH1 was taken up by macrophages in the liver, bone marrow, and spleen via receptor-mediated endocytosis from plasma; therefore, it cannot be excluded that the distributions of MDH1 in these tissues are a result of plasma clearance of the enzyme by macrophages rather than energy metabolism [Bijsterbosch et al., 1983].

In general, the overall tissue distribution of MDH1 tightly correlates with the location of human $NADP^+$-malic enzyme ($NAPD^+$-ME, EC 1.1.1.40). Its distribution implies that sufficient reducing equivalents of NADPH, functioning as a surrogate of NADH, are crucial for an active oxidative metabolism in contractile muscles, and for detoxification of reactive oxygen radicals generated during respiration; however, only NADPH is important in β-hydroxylation reactions during steroid hormone synthesis [Loeber et al., 1994]. We speculate that the genes regulating the process of anaplerosis (increasing total TCA cycle pool size and NADH production), which is coordinated by MDH2 and $NADP^+$-ME, localize in the same oxidative cellular environments. The two MDH isozymes work co-operatively in the malate-aspartate shuttle to transport across the mitochondrial membrane and they may express in the same cellular locations. Our observation of MDH1 in astrocytes is consistent with prior data describing MDH2 found in the astrocytes of neonatal rat brains [Oh et al., 1991]. This suggests that astrocytes may be a primary location for oxidative metabolism in the brain.

The expression of the enzymes of glucose utilization and fatty acid β-oxidation pathways is tissue-specific and developmentally regulated [Barger and Kelly, 1999]. The pattern of MDH1 mRNA expression is correlated with changes in energy metabolism during development. In the mammalian fetal heart and liver, glucose serves as the major energy source for more oxygen-efficient ATP production in a relatively hypoxic environment. Postnatally, the main energy metabolism shifts to fatty acid β-oxidation, allowing large capacity ATP production at high oxygen consumption rates [Warshaw, 1972; Momma et al., 1987; Barger and Kelly, 1999]. Here, we report that MDH1 is

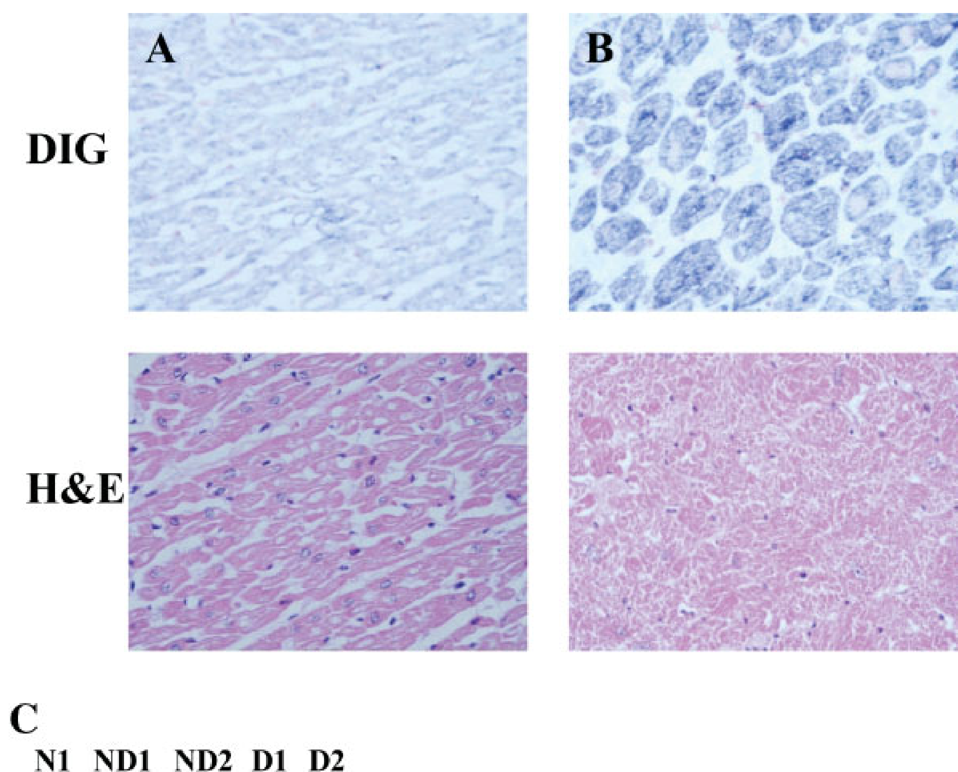


Fig. 3. Over-expression of MDH1 in dilated cardiomyopathy (DCM). A representative in situ hybridization result indicated MDH1 mRNA expression in (**A**) normal human adult heart and (**B**) DCM heart. Methods as in Figure 1. **C**: Western blot of MDH1 protein in left ventricular heart tissues. **Lane 1**: Normal subject N1, (**lanes 2** and **3**) non-dilated cardiomyopathy (non-DCM) patients ND1 and ND2, and (**lanes 4** and **5**) DCM patients D1 and D2.

initially expressed in the heart at the 3rd month and reaches full expression from the 5th month of development, but then declines postnatally to a still-high level from infancy through adulthood. A similar pattern of increase in MDH2 expression was noted in developing rat heart and liver followed by a decline during the first postnatal week [Warshaw, 1972; Momma et al., 1987; Barger and Kelly, 1999]. A rapid transition toward fatty acid β-oxidation through the increasing activity of carnitine palmitoyl transferase 1 (CPT-1) is found in rat heart during the early postnatal period [Warshaw, 1972]. Correspondingly, it has been reported that MDH1 and MDH2 increased twofold until birth in developing rabbit liver and then declined after birth [Smith, 1970]. This reflects that the increased expression of MDHs is required for the high demands of energy metabolism in developing tissues [Ding et al., 1994]. The capacity of the NADH shuttle declines postnatally due to the decreasing expression of the oxoglutarate/malate carrier (OMC) [Scholz et al., 1998; Rupert et al., 2000]. In addition, glucose is the major energy substrate for the tissues of the brain throughout development and in maturity, thus there is relatively high TCA cycle activity with limited fatty acid β-oxidation [Kelly et al., 1989].

Dilated cardiomyopathy (DCM) is a heart-muscle disease characterized by ventricular dilation, cardiac hypertrophy, and decreased systolic contraction. It is the most common form of cardiomyopathy accounting for approximately 60% of all cases of cardiomyopathy and is a major indication for cardiac transplantation [Durand et al., 1995]. Approximately 20% of cases are inherited and X-linked dilated cardiomyopathies have been reported to be related to mutations in the dystrophin gene [Michels et al., 1992; Towbin et al., 1993]. In the relatively oxygen-starved environment of hypertrophic

and failing heart tissues, the terminally differentiated myocardium adapts to increasing pressure overload by an increase in protein content per myocardial cell [Chien et al., 1991; Barger and Kelly, 1999; Razeghi et al., 2001]. During this process, the cardiac substrate utilization reverts from a primary usage of fatty acids to a pattern more common in fetuses, which primarily use glucose and lactate as substrates to maximize oxygen-efficient production of ATP but at the cost of reduced contractile reserve [Barger and Kelly, 1999; Rupert et al., 2000]. Molecular markers of myocardial hypertrophy including atrial natriuretic factor (ANP), skeletal α-actin, and β-myosin heavy chain (MHC) belong to embryonic genes. Similarly, over-expression of proteins such as myosin heavy chain, rae28 and PGC-1 were found to be expressed predominantly in the embryonic stage [Lehman et al., 2000; Koga et al., 2002]. PGC-1 was reported to play a regulatory role in mitochondrial energy production and fatty acid synthesis [Lehman et al., 2000]. Several genes related to transcription factors, embryonic genes and contractile proteins that are typically expressed exclusively in the fetus are activated in DCM [Chien et al., 1991; Razeghi et al., 2001]. Reduced expression of a key fatty acid β-oxidation enzyme medium-chain acyl-Co-enzyme A dehydrogenase (MCAD) and long-chain fatty acyl-Co A transporter CPT-1 is also found [Sack et al., 1996] and the expression of those genes has been shown to be modulated by upstream promoters in response to postnatal and hypertrophic stimuli [Barger and Kelly, 1999].

On the other hand, the mechanisms of increased glycolytic and oxidative activity in hypertrophic and failing heart are not well-characterized and variable degrees of corresponding enzyme activities have been recorded. It has been shown that levels of hexokinase and glyceraldehydes-3-phosphate dehydrogenase are increased in hypertrophied myocytes, while phosphofructokinase and pyruvate kinase levels have been found to remain the same or decrease [Mavrides and Korecky, 1985; Klein et al., 1986; Taegtmeyer and Overturf, 1988; Do et al., 1997; Takeuchi et al., 1998]. It has been proposed that the rate-limitation of glycolysis is determined by glucose transport at the cell membrane. A shift of the glucose transporter isoforms from adult isoform GLUT4 to fetal isoform GLUT1 has been found to accelerate glucose transport into cardiac myocytes [Montessuit and Thorburn, 1999; Razeghi et al., 2001].

While it has been reported that MDH2 is unchanged in hypertrophied heart, another report suggested that total aspartate aminotransferase (AAT) and soluble MDH protein was increased by 20% in aortic constriction induced hypertrophy in rat after 3 months. Another citation found the downregulation of MDH family protein expression in the right atrium of DCM patients [Knecht et al., 1994]. Since the degree of susceptibility to hypoxia may be significantly different between the right atrium and the highly dilated left ventricle of our study, those results are unlikely to be relevant to our work. In contrast, the patients with affected aortic valve diseases and mitral valve diseases without hypertrophy have a high fatty acid oxidation activity with significantly lower TCA cycle activity, as is indicated by an observed increase in the activities of 1–3-hydroxyacyl-CoA dehydrogenase (HADH) and reducing citrate synthase (CS) [Klein et al., 1986].

By designing a cDNA probe and showing the morphological staining of hypertrophied muscle, we have demonstrated the upregulation of MDH1 expression in DCM patients but not in valve-diseased non-DCM patients and normal individuals. This is physiologically adaptive in that it enhances the capacity of glycolytic oxidation in a relatively hypoxic environment to satisfy the high-energy demand in DCM. We postulate that this shift towards glycolytic oxidation is unique to DCM.

Localization studies indicate that the expression of MDH1 involved in cellular energy metabolism is regulated in a tissue-specific manner. Over-expression of MDH1 in fetal and DCM heart could be adaptive to support the production of adequate ATP to maintain function in relatively hypoxic environments. Analysis of the molecular regulation controlling energy metabolic switch during the development of the developing and hypertrophied heart will be further explored.

ACKNOWLEDGMENTS

We thank Ms. Carmen Chan and Dr. Hiu-Ming Li for their assistance in the collection of the heart samples. We also thank Mr. David Cook, Mr. Patrick Tik-Wan Law,

and Dr. Richard P. Junghans for their helpful discussion of the manuscript.

REFERENCES

Aziz LE, Chien SM, Patel HV, Freeman KB. 1981. A putative precursor of rat liver mitochondrial malate dehydrogenase. FEBS Lett 133:127–129.

Barger PM, Kelly DP. 1999. Fatty acid utilization in the hypertrophied and failing heart: Molecular regulatory mechanisms. Am J Med Sci 318:36–42.

Bijsterbosch MK, Duursma AM, Bouma JM, Gruber M. 1983. Plasma clearance and endocytosis of cytosolic malate dehydrogenase in the rat. Biochem J 210:419–428.

Chien KR, Knowlton KU, Zhu H, Chien S. 1991. Regulation of cardiac gene expression during myocardial growth and hypertrophy: Molecular studies of an adaptive physiologic response. FASEB J 5:3037–3046.

Ding SH, Choudhury BK, Setoyama C, Shimada K. 1994. Two regulatory elements in the 5′ region of the mouse mitochondrial malate dehydrogenase gene. Biochem Mol Biol Int 32:131–138.

Do E, Baudet S, Verdys M, Touzeau C, Bailly F, Lucas-Heron B, Sagniez M, Rossi A, Noireaud J. 1997. Energy metabolism in normal and hypertrophied right ventricle of the ferret heart. J Mol Cell Cardiol 29:1903–1913.

Durand JB, Abchee AB, Roberts R. 1995. Molecular and clinical aspects of inherited cardiomyopathies. Ann Med 27:311–317.

Grant PM, Roderick SL, Grant GA, Banaszak LJ, Strauss AW. 1987. Comparison of the precursor and mature forms of rat heart mitochondrial malate dehydrogenase. Biochemistry 26:128–134.

Hall MD, Levitt DG, Mcallister-Henn L, Banaszak LJ. 1991. Purification and crystallization of recombinant escherichia coli malate dehydrogenase. J Mol Biol 220: 551–553.

Hartmann CM, Gehring H, Christen P. 1993. The mature form of imported mitochondrial proteins undergoes conformational changes upon binding to isolated mitochondria. Eur J Biochem 218:905–910.

Joh T, Takeshima H, Tsuzuki T, Setoyama C, Shimada K, Tanase S, Kuramitsu S, Kagamiyama H, Morino Y. 1987a. Cloning and sequence analysis of cDNAs encoding mammalian cytosolic malate dehydrogenase. Comparison of the amino acid sequences of mammalian and bacterial malate dehydrogenase. J Biol Chem 262: 15127–15131.

Joh T, Takeshima H, Tsuzuki T, Shimada K, Tanase S, Morino Y. 1987b. Cloning and sequence analysis of cDNAs encoding mammalian mitochondrial malate dehydrogenase. Biochemistry 26:2515–2520.

Kelly DP, Gordon JI, Alpers R, Strauss AW. 1989. The tissue-specific expression and developmental regulation of two nuclear genes encoding rat mitochondrial proteins. Medium chain acyl-CoA dehydrogenase and mitochondrial malate dehydrogenase. J Biol Chem 264:18921–18925.

Klein HH, Spaar U, Schlepple H, Wiegand V, Kreuzer H. 1986. Comparative analysis of myocardial enzyme activities of the energy-supplying metabolism in patients with dilative cardiomyopathies and valve diseases. Clin Cardiol 9:197–202.

Knecht M, Regitz-Zagrosek V, Pleissner KP, Jungblut P, Steffen C, Hildebrandt A, Fleck E. 1994. Characterization of myocardial protein composition in dilated cardiomyopathy by two-dimensional gel electrophoresis. Eur Heart J 15:D37–D44.

Koga H, Kaji Y, Nishii K, Shirai M, Tomotsune D, Osugi T, Sawada A, Kim JY, Hara J, Miwa T, Yamauchi-Takihara K, Shibata Y, Takihara Y. 2002. Overexpression of polycomb-group gene *rae28* in cardiomyocytes does not complement abnormal cardiac morphogenesis in mice lacking rae28 but causes dilated cardiomyopathy. Lab Invest 82:375–385.

Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. 2000. Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. J Clin Invest 106:847–856.

Liew CC, Hwang DM, Fung YW, Laurenssen C, Cuckermen E, Tsui S, Lee CY. 1994. A catalogue of genes in the cardiovascular system as identified by expressed sequence tags. Proc Natl Acad Sci USA 91:10645–10649.

Lo AS, Liew CT, Law PT, Garcia-Barcelo M, Tsui SK, Fung KP, Lee CY, Waye MM. 1999. Radiation hybrid mapping of human cytosolic malate dehydrogenase (hcMDH) to the short arm of chromosome 2. Somat Cell Mol Genet 25:109–113.

Loeber G, Maurer-Fogy I, Schwendenwein R. 1994. Purification, cDNA cloning and heterologous expression of the human mitochondrial NADP(+)-dependent malic enzyme. Biochem J 304:687–692.

Mavrides C, Korecky B. 1985. Subcellular distribution of the enzymes of the malate-aspartate shuttle in rat heart and effect of experimental cardiac hypertrophy. Biosci Rep 5:95–100.

Michels VV, Moll PP, Miller FA, Tajik AJ, Chu JS, Driscoll DJ, Burnett JC, Rodeheffer RJ, Chesebro JH, Tazelaar HD. 1992. The frequency of familial dilated cardiomyopathy in a series of patients with idiopathic dilated cardiomyopathy. N Engl J Med 326:77–82.

Momma K, Takao A, Ito R, Nishikawa T. 1987. In situ morphology of the heart and great vessels in fetal and newborn rats. Pediatr Res 22:573–580.

Montessuit C, Thorburn A. 1999. Transcriptional activation of the glucose transporter GLUT1 in ventricular cardiac myocytes by hypertrophic agonists. J Biol Chem 274:9006–9012.

Nishiyama M, Matsubara N, Yamamoto K, Iijima S, Uozumi T, Beppu T. 1986. Nucleotide sequence of the malate dehydrogenase gene of Thermus flavus and its mutation directing an increase in enzyme activity. J Biol Chem 261:14178–14183.

Oh YJ, Markelonis GJ, Oh TH. 1991. Immunocytochemical localization of mitochondrial malate dehydrogenase in primary cultures of rat astrocytes and oligodendrocytes. J Histochem Cytochem 39:681–688.

Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, Taegtmeyer H. 2001. Metabolic gene expression in fetal and failing human heart. Circulation 104:2923–2931.

Rupert BE, Segar JL, Schutte BC, Scholz TD. 2000. Metabolic adaptation of the hypertrophied heart: Role of the malate/aspartate and alpha-glycerophosphate shuttles. J Mol Cell Cardiol 32:2287–2297.

Sack MN, Rader TA, Park S, Bastin J, Mccune SA, Kelly DP. 1996. Fatty acid oxidation enzyme gene expression is

downregulated in the failing heart. Circulation 94:2837–2842.
Scholz TD, Koppenhafer SL, Teneyck CJ, Schutte BC. 1998. Ontogeny of malate-aspartate shuttle capacity and gene expression in cardiac mitochondria. Am J Physiol 274: C780–C788.
Smith CH. 1970. Perinatal regulation of mitochondrial and cytoplasmic enzymes in rabbit liver. Pediatr Res 4:328–336.
Stuewe SR, Gwirtz PA, Agarwal N, Mallet RT. 2000. Exercise training enhances glycolytic and oxidative enzymes in canine ventricular myocardium. J Mol Cell Cardiol 32:903–913.
Taegtmeyer H, Overturf ML. 1988. Effects of moderate hypertension on cardiac function and metabolism in the rabbit. Hypertension 11:416–426.
Takekura H, Yoshioka T. 1990. Different metabolic responses to exercise training programmes in single rat muscle fibres. J Muscle Res Cell Motil 11:105–113.
Takeuchi K, Mcgowan FX, Jr., Glynn P, Moran AM, Rader CM, Cao-Danh H, Del Nido PJ. 1998. Glucose transporter upregulation improves ischemic tolerance in hypertrophied failing heart. Circulation 98:II234–II241.
Tanaka T, Inazawa J, Nakamura Y. 1996. Molecular cloning and mapping of a human cDNA for cytosolic malate dehydrogenase (MDH1). Genomics 32:128–130.
Towbin JA, Hejtmancik JF, Brink P, Gelb B, Zhu XM, Chamberlain JS, Mccabe ER, Swift M. 1993. X-linked dilated cardiomyopathy. molecular genetic evidence of linkage to the duchess muscular dystrophy (dystrophin) gene at the Xp21 locus. Circulation 87:1854–1865.
Warshaw JB. 1972. Cellular energy metabolism during fetal development. IV. Fatty acid activation, acyl transfer and fatty acid oxidation during development of the chick and rat. Dev Biol 28:537–544.
Webb LE, Hill EJ, Banaszak LJ. 1973. Conformation of nicotinamide adenine dinucleotide bound to cytoplasmic malate dehydrogenase. Biochemistry 12:5101–5109.