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MOLECULAR CLONING AND GENE MAPPING OF HUMAN BASIC AND ACIDIC CALPONINS

Motofumi Maguchi, Wataru Nishida, Katsuhiko Kohara, Akira Kuwano,†
Ikuko Kondo,† and Kunio Hiwada*

The Second Department of Internal Medicine, †Department of Hygiene, Ehime University School of Medicine, Shigenobu, Onsen-gun, Ehime 791-02, Japan

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SUMMARY: The nucleotide and deduced amino acid sequences of human basic and acidic calponins were determined. The basic calponin cDNA from human aorta (1496 bp) contained a single open reading frame (ORF) which encodes 297 amino acids (33,169 Da). The acidic calponin cDNA from human kidney (1607 bp) contained a single ORF which encodes 329 amino acids (36,412 Da). Basic calponin mRNA was expressed in only smooth muscle tissues, but acidic calponin mRNA was expressed in non-smooth muscle tissues as well as smooth muscle tissues. Fluorescent in situ hybridization revealed that basic and acidic calponin genes localize in 19p13.1-13.2 and 1p21-22 of human chromosomes, respectively.

In response to specific stimuli, vascular smooth muscle cells (VSMCs) convert from a contractile phenotype (differentiated) to a synthetic phenotype (dedifferentiated). This phenotypic modulation is a key event in atherosclerosis and in restenosis of coronary arteries after balloon angioplasty. During the dedifferentiation of VSMCs, a number of cytoskeletal proteins, such as α-actin, myosin heavy chain isoforms SM1 and SM2, desmin, meta-vinculin, caldesmon (CaD) , and calponin have been shown to be lost [1-3]. Similar down regulation of the cytoskeletal proteins has been also confirmed in cell culture. Gimona et al. [4] reported that cultivation of gizzard smooth muscle cells in vitro resulted in a loss of calponin and meta-vinculin accompanied by a transition of caD isoforms from heavy CaD to light CaD [5]. CaD and calponin are two major proteins which

* To whom correspondence should be addressed. Fax: +81-899-64-1923.

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interact with actin-tropomyosin filament and are shown to inhibit actin-activated myosin Mg-ATPase activity in vitro [6-8]. The deduced polypeptide of calponin from rat aorta is markedly basic (estimated isoelectric point (pI) of 8.74) and contains unique three tandem repeats [9-10]. Recently, another homologue of calponin, acidic calponin, has been identified in rat aorta. The amino terminus of acidic calponin is highly homologous to basic calponin, but it has marked acidic domain at the carboxyl terminus [11]. The physiological role of acidic calponin is uncertain, however, the transition of two isoforms of calponin may take place during phenotypic modulation of VSMCs like as CaD.

In this study, we isolated basic and acidic calponin cDNAs from human tissues and identified independent chromosomal localization of the human calponin genes. We also confirmed tissue specific expression of basic and acidic calponins in a variety of human tissues.

METHODS

Preparation of radio labeled calponin cRNA probes: Basic calponin cDNA from rat aorta was prepared as described previously [10]. Rat acidic calponin cDNA was cloned as follows. First-strand cDNA was synthesized from total RNA of rat aorta and was subjected to PCR amplification. The sense primer corresponds to 227-246 bp (5'-CAGCCATGACCCACTTCAAC-3'), and antisense primer corresponds to 1193-1212 bp (5'-GCCCTGGTCGTCACCATACT-3') of the rat acidic calponin cDNA [11]. The amplified cDNA fragment was subcloned into pBluescript II SK (+) vector (Stratagene, La Jolla, CA, USA). [γ-^{32}P] labeled calponin cRNA probes were prepared using AmpliScribe Transcription Kit (Epincentre Technologies, Madison, USA).

Molecular cloning of basic and acidic calponin cDNAs from human tissues: We screened human aorta and kidney cDNA libraries constructed in λgt 10 (CLONTECH Laboratories, Inc, Palo Alto, CA, USA) using the radio labeled rat aorta calponin cRNA probes. We isolated positive plaques, excised inserts and subcloned the cDNA fragments into pBluescript II SK (+) vector. Deletion mutants were prepared using Discrete deletion kit (Epincentre). Sequencing reaction was carried out using AutoRead Sequencing kit (Pharmacia LKB Biotechnology, Uppsala, Sweden), and the products were analyzed on A.L.F. DNA Sequencer (Pharmacia).

Northern blot analysis: [α-^{32}P] labeled human calponin cDNA probes were prepared using Prime It II Random Primer Labeling kit (Stratagene). Northern blot membranes of multiple human tissues (CLONTECH) were hybridized with the probes.

Fluorescent in situ hybridization: We performed chromosomal localization of human basic and acidic calponins to determine whether they are encoded by different genes or not. Cloned human basic and acidic calponin cDNAs were

biotin-labeled, ethanol precipitated with human placental DNA, and resuspended in hybridization solution (50% formamide/ 2XSSC/ 10% dextran sulfate). Chromosome slides were prepared from PHA-stimulated female peripheral lymphocytes cultures treated with thymidine synchronization and bromodeoxyuridine. Direct R-banding fluorescence in situ hybridization and sequential G-banding by Wright Giemsa staining were performed as described in detail elsewhere [12].

RESULTS

We screened $7.5X10^5$ of plaques in human aorta cDNA library using basic calponin probe cloned from rat aorta and obtained 20 positive clones. Sequencing of 4 clones revealed that human basic calponin cDNA was 1496 bp in length and contained a single ORF which encodes 297 residues with a calculated molecular weight of 33169 Da and a pI of 9.4. We also screened $2.6X10^8$ of plaques in human kidney cDNA library using acidic calponin probe cloned from rat aorta and obtained 4 positive clones. Sequencing of all clones revealed that human acidic calponin cDNA was 1607 bp in length and contained a single ORF which encodes 329 residues with a calculated molecular weight of 36412 Da and a pI of 5.64. The primary structures of human basic and acidic calponins are shown in Fig. 1. Both isoforms of human calponin contained similar three tandem repeats which is also observed in rat and chicken gizzard basic calponin. The first 273 amino acids of basic and acidic calponins are highly homologous (71% identity) and this portion is markedly basic (calculated pI of 9.76 and 9.44, respectively). The acidity of human acidic calponin is resulted from the 58 residues at the carboxyl terminus which have a predicted pI of 3.51.

Fig. 2 illustrates the result of Northern blot analysis for the determination of the tissue distribution of human basic and acidic calponin mRNAs. Basic

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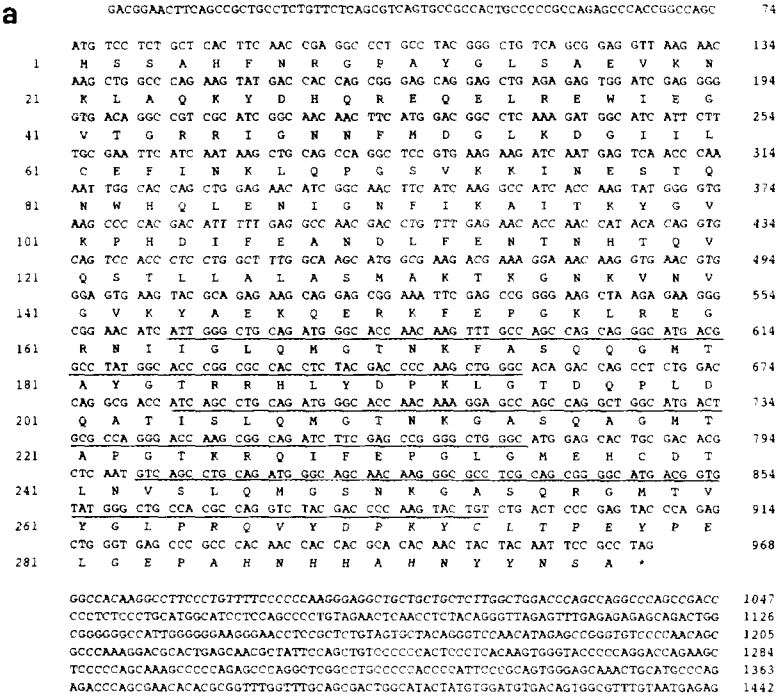
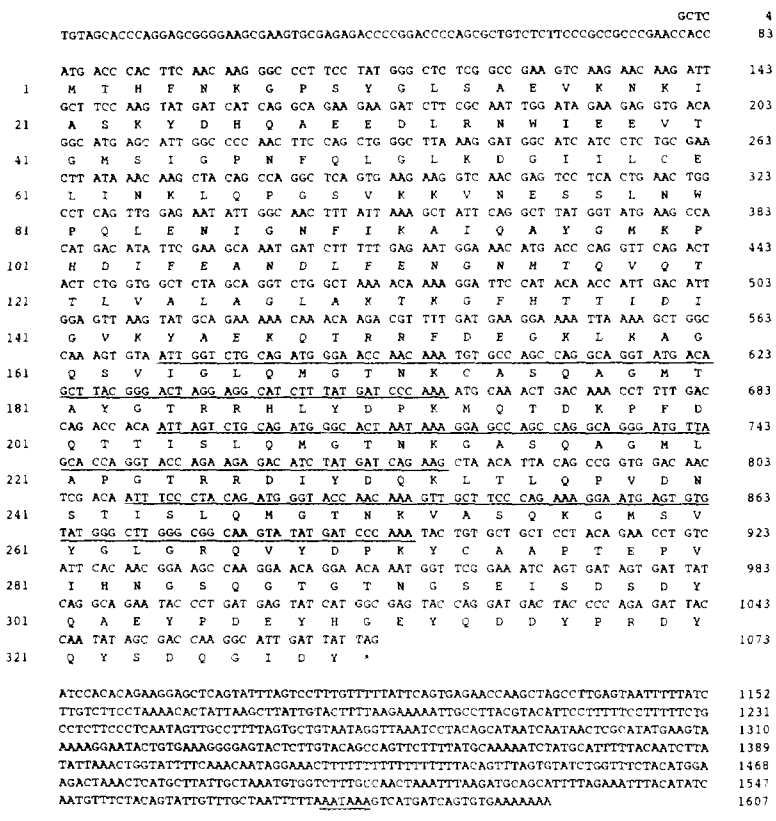
Figure 1. (a) Nucleotide and deduced amino acid sequences of human basic calponin. The underlining identifies the three repeated motif and the putative polyadenylation signal (AATAAA). Left and right columns denote sequence numbers of amino acids and nucleotides, respectively.

(b) Nucleotide and deduced amino acid sequences of human acidic calponin. The underlining identifies the three repeated motif and the putative polyadenylation signal (AATAAA). Left and right columns denote sequence numbers of amino acids and nucleotides, respectively.

a



b



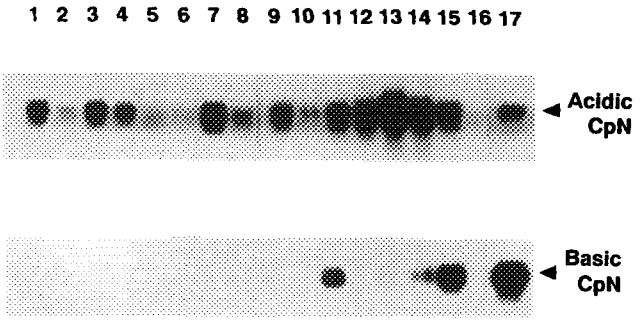


Figure 2. Northern blot analysis of basic and acidic calponin mRNAs in human tissues. Lanes 1-16 contain 2μg of poly(A) RNA, and lane 17 contains 0.5μg of poly(A) RNA. (1) heart, (2) brain, (3) placenta, (4) lung, (5) liver, (6) skeletal muscle, (7) kidney, (8) pancreas, (9) spleen, (10) thymus, (11) prostate, (12) testis, (13) ovary, (14) small intestine, (15) colon, (16) peripheral blood leukocyte, and (17) aorta.

calponin was specifically expressed in only smooth-muscle tissues (prostate, testis, ovary, small intestine, colon, and aorta), while acidic calponin was expressed in both of smooth-muscle and non smooth-muscle tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and aorta). In acidic calponin, blot pattern was doublet in all tissues examined. The result suggests that at least two sub-isoforms of acidic calponin exist in human tissues.

Of 50 metaphases examined for basic calponin, two showed symmetrical double spots at both homologous 19p13.1-13.2, and another 10 metaphases showed double spots on single chromosome 19p13.1-13.2. All double spots and 86% of the single spot detected were on chromosome 19p13.1-13.2. We therefore assigned basic calponin gene to chromosome 19p13.1-13.2. They were further confirmed in G-banded chromosomes (Fig.3). Of 50 metaphases examined for acidic calponin, 11 metaphases showed double spots on a single chromosome 1p21-22 (data not shown). All double spots except 80% of the single spot detected were on chromosome 1p21-22. We therefore assigned acidic calponin gene to chromosome 1p21-22. They were further confirmed in G-banded chromosomes.

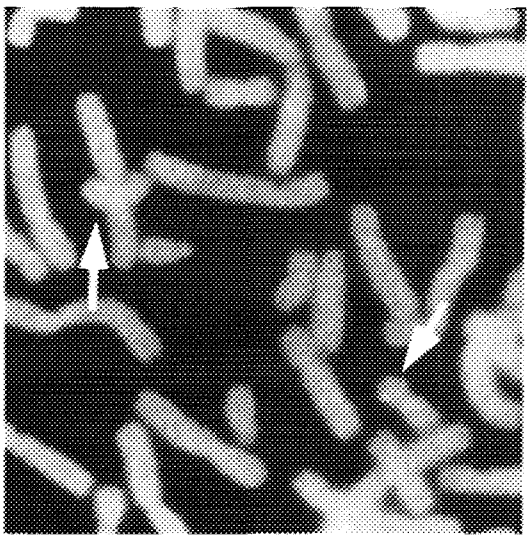


Figure 3. Chromosomal localization of human basic calponin gene. Arrows indicate the positive fluorescence signals observed on the human chromosome 19p13.1-13.2.

DISCUSSION

The primary structures of human basic and acidic calponins were presented. Furthermore we demonstrated that the basic and acidic calponins did not arise via alternative splicing but were encoded by different genes. Either isoform of human calponins contained three repeated motif, which is well preserved between chick, rat, and human. It has been reported that unphosphorylated basic calponin binds to actin to inhibit the actin-activated myosin Mg-ATPase in vitro [6-8]. This inhibition is abolished by phosphorylation of serine and/or threonine residues in the repeated motif by protein kinase C [13-14]. These findings indicate that basic calponin modulates contraction of smooth muscle cells through the phosphorylation by protein kinase C. Basic calponin is specific for smooth muscle, and their expression is dependent on the differential state of smooth muscle cells. Gimona et al. [4] demonstrated a differentiation-linked increase in basic calponin production in embryonic chicken gizzard and a down regulation of basic calponin during cultivation of gizzard smooth muscle cells in vitro. We also demonstrated an increase in basic calponin mRNA in rat aorta during the development [10]. By contrast, acidic calponin is markedly

expressed in cultured cells [11], and is expressed in non-smooth muscle tissues as well as smooth muscle tissues. The physiologic role of acidic calponin is uncertain, however its abundant expression in non-smooth muscle tissues is suggestive for the association with cytoskeletal component rather than contractile involvement. Finally, our data clearly show that tissue and differential-state specific expression of basic and acidic calponins results from different transcriptional regulation on independent chromosomal loci.

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