Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 353 (2005) 31 – 40

GENE
SECTION
FUNCTIONAL GENOMICS

www.elsevier.com/locate/gene

Characterization and functional significance of myotrophin: A gene with multiple transcripts☆

Gautam Adhikary[1,2], Sudhiranjan Gupta[1], Parames Sil[3], Yasser Saad, Subha Sen*

Department of Molecular Cardiology, NB 50, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, United States

Received 1 December 2004; received in revised form 7 March 2005; accepted 22 March 2005

Received by J.A. Engler

Abstract

The underlying mechanism for the development of cardiac hypertrophy that advances to heart failure is not known. Many factors have been implied to play a role in this process. Among others, we have isolated and identified myotrophin, a factor that stimulates myocytes growth, from spontaneously hypertensive rat (SHR) heart and patients with dilated cardiomyopathy. The gene encoding myotrophin has been cloned and expressed in *E. coli*. Recently, myotrophin gene has been mapped and shown to be a novel gene localized in human chromosome 7q-33. To define the characteristics of each transcript and its pathophysiological significance, we examined transcripts of myotrophin in SHR heart during progression of hypertrophy. Northern blot analysis of myotrophin mRNA showed multiple transcripts. We isolated and characterized various myotrophin cDNA clones corresponding to the multiple transcripts by 5′ "stretch plus" rat heart cDNA library screening. Sequence analysis of these cDNA clones indicates that each clone has a unique 5′ UTR and multiple 3′ UTR with varying lengths, repeated ATTTA motifs and many polyadenylation signals. In vitro transcripts generated from all these myotrophin-specific cDNA clones translate in vitro to a 12-kD protein. Among pathophysiological significance, we determined mRNA expression in 9 days old, 3 weeks old and 31 weeks old and observed a linear increased during the progression of hypertrophy. In WKY, this mRNA level remained the same throughout the growth and development of hypertrophy. Our data strongly suggest that myotrophin appears to be a candidate gene for cardiac hypertrophy and heart failure.

Abbreviations: SHR, spontaneously hypertensive rat; WKY, Wisytar Kyto; cDNA, DNA complimentary to RNA; UTR, untranslated region; kD, kilo dalton; mRNA, messenger RNA; DCM, dilated cardiomyopathy; ANF, atrial natriuretic factor; β-MHC, β-myosin heavy chain; PKC, protein kinase C; DOCA-salt, deoxycorticosterone acetate salt hypertensive rat; NF-κB, nuclear factor kB; IκBα, inhibitory molecule of NF-κB; EDTA, ethylene diamine tetra acetic acid; SDS, sodium dodicyl sulphate; SSC, standard sodium citrate; SSPE, standard sodium phosphate EDTA; kb, kilo base; kbp, kilo base pair; ORF, open reading frame; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; MTPN, myotropihin; NCBI, national center for biotechnology information; SNPs, single nucleotide polymorphism (s); MAS, myotrophin antisense primer; LL, lambda left primer; CPSF, cleavage and polyadenylation specific factor; ARE, adenylate and uridylate-rich element; AUF1, adenylate/uridylate (AU) rich RNA-binding factor 1; HUR, Hu antigen R; AUH, AU binding homolog of enoyl-CoA hydrase.

☆ The nucleotide sequence(s) reported in this paper has been submitted to the GenBank Data Bank with accession number(s) AY951952.

* Corresponding author. Tel.: +1 216 444 2056; fax: +1 216 444 3110.
E-mail address: sens@ccf.org (S. Sen).

[1] Both authors participated equally in generating the data.

[2] Current address: Case Western Reserve University, Cleveland, OH, United States.

[3] Current address: Department of Chemistry, Bose Institute, Calcutta, India.

0378-1119/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2005.03.045

1. Introduction

Cardiac hypertrophy associated with hypertension and subsequent heart failure is the most leading cause of death in developed countries with diverse clinical and pathological manifestations (Maron et al., 1995; Maron, 1997). Unfortunately, the underlying mechanism for the molecular changes that take place during this progression process is not known. Studies from our laboratory have demonstrated that mechanism involved in the initiation or regression of hypertrophy in spontaneously hypertensive rats (SHR) cannot be fully explained as response to blood pressure control alone. We hypothesized that the development of hypertrophy is initiated by a humoral or mechanical signal to the myocardium, which in turn produces a soluble factor that triggers protein synthesis and initiates myocardial growth. In exploring the factor that initiates cardiac hypertrophy, our laboratory has identified a factor, myotrophin, from SHR and DCM human hearts (Sen et al., 1990; Sil et al., 1993) that stimulates myocytes growth. Myotrophin has been shown to stimulate (a) incorporation of [^{3}H] leucine as well as [^{14}C] phenylalanine into myocyte protein in vitro, (b) cell growth and (c) the increase in cell surface area in a dose-dependent fashion compared with controls (Sen et al., 1990). Neonatal myocytes treated with myotrophin displayed an accelerated myofibrillar growth and an organization into sarcomeres (Sen et al., 1990). Myotrophin has also been shown to be specific for myocytes only, because it has no effect on fibroblasts, endothelial cells, or smooth muscle cells. It was observed that myotrophin selectively increased the transcript level of the proto-oncogenes c-*myc*, c-*fos* and c-*jun*, along with connexin (gap junction protein), atrial natriuretic factor (ANF) and β-myosin heavy chain (β-MHC) gene in cultured myocytes (Mukherjee et al., 1993). Furthermore, increased level of myotrophin has also been correlated with the onset of hypertrophy in SHR and in humans (Sil et al., 1995). Myotrophin gene was mapped, for the first time, to human chromosome 7q33 (Mitra et al., 2001). Moreover, data from our laboratory also showed that protein synthesis stimulatory activity of myotrophin action was mediated through PKC signaling pathway (Sil et al., 1998). Most recently, we examined myotrophin gene in three different hypertensive models, e.g. renal hypertension, DOCA-salt and aortic coarctation where myotrophin levels were elevated (Sil et al., 2004). To further evaluate the physiological relevance of myotrophin in vivo, we used α-myosin heavy chain promoter to generate transgenic mice in which myotrophin is overexpressed in the heart (Sarkar et al., 2004). Overexpression of myotrophin specifically in the myocardium resulted in severe cardiac hypertrophy that advanced to heart failure. These mice exhibited left ventricular hypertrophy, atrial dilation, myocyte necrosis, multiple focal fibrosis, pleural effusion and compromised cardiac function associated with significant reduction in ejection fraction and fractional shortening (Sarkar et al., 2004). All our findings provided a wealth of convincing evidence that myotrophin plays an integral role in initiating cardiac hypertrophy.

The gene coding for myotrophin has been cloned and expressed in *E. coli* (Sivasubramanian et al., 1996). The recombinant myotrophin showed the same biological activity as native myotrophin. Sequence analysis of the cDNA clone revealed that myotrophin consists of 118 amino acids and contains two full and two half-ankyrin repeats (Sivasubramanian et al., 1996). Interestingly, one of the ankyrin repeat showed structural homology with IκBα, an inhibitory molecule of NF-κB. In addition, structural analysis also revealed the putative phosphorylation sites for PKC and casein kinase II (Sivasubramanian et al., 1996). Myotrophin has multiple transcripts in the rat heart. Among these transcripts, the 4.3-kb transcripts are present in abundance, but the levels of the other myotrophin transcripts in mRNA are relatively low. The complete profile of the transcripts corresponding to each clone and also the structural organization of myotrophin gene has not been established. To define the significance of each myotrophin transcript, we describe (1) the isolation and characterization of different myotrophin cDNA clones corresponding to their multiple transcripts from the rat heart 5′ "stretch-plus" cDNA library (2) the organization of the myotrophin gene, and (3) the identification of different myotrophin mRNAs in SHR heart by northern hybridization.

2. Materials and methods

2.1. Materials and methods

All male SHRs were obtained from Taconic Farms, Germantown, NY. Timed pregnant rats were obtained from Hilton Farm, Scottsdale, PA. All animals were kept in accordance with NIH and Institutional guidelines. The rats were housed three per cage and maintained under controlled conditions of light, temperature and humidity. All rats had free access to water and food. Hearts were removed after ether anesthesia. Atria and blood vessels were removed. Hearts were then blotted dry and immediately frozen to −70 °C.

2.2. Northern analysis of myotrophin mRNAs

Total RNA was isolated from 9-day-old SHR hearts following the phenol chloroform extraction method (Chomczynski and Sacchi, 1987). Poly (A) enriched RNA was isolated using the oligo(dT)-cellulose type-III (Collaborative Research, Bedford, MA) column at high-salt conditions (10 mM Tris–HCl, pH 7.5, 1 mM EDTA, 0.5 M NaCl). After washing the column with high-salt buffer, poly (A) RNA was eluted with no-salt (10 mM Tris–HCl, pH 7.5, 1 mM EDTA) buffer. The poly (A) RNA eluted by this method was fractionated on 1% agarose formaldehyde gels, transferred to a zeta probe membrane (BIORAD,