Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Biochemical and Biophysical Research Communications 333 (2005) 344–352
BBRC
www.elsevier.com/locate/ybbrc

A novel six-transmembrane protein hhole functions as a suppressor in MAPK signaling pathways ☆

Junmei Zhou [1], Yongqing Li [1], Pei Liang, Wuzhou Yuan, Xiangli Ye, Chuanbing Zhu, Yingduan Cheng, Yuequn Wang, Guan Li, Xiushan Wu *, Mingyao Liu *

The Center for Heart Development, College of Life Sciences, Hunan Normal University, Changsha, 410081 Hunan, People's Republic of China

Received 9 May 2005
Available online 31 May 2005

Abstract

Src homology 3 (SH3) domains mediate intracellular protein–protein interactions through the recognition of proline-rich sequence motifs on cellular proteins. Such protein–protein interactions can activate the protein kinase cascade that mediates MAPK signaling pathway. The human *hole* gene, *hhole*, is a 319-amino acid six-transmembrane protein with proline-rich C-terminal motifs and N-terminal ERK binding domains (D-domains). The hhole protein is highly conserved in evolution across different species from *elegent*, mouse to human. Northern blot analysis indicates that *hhole* is expressed in heart, liver, skeletal muscle, and pancreas at adult stages and in most of the examined embryonic tissues, especially at a higher level in heart. Using a GFP-labeled hhole protein, we demonstrate that hhole is localized in plasma membrane or proximal region of the membrane. Overexpression of hhole in COS-7 cells strongly inhibited the transcriptional activities of AP-1 and SRE while deletion of the C-terminal proline-rich motifs or the N-terminal ERK binding D-domain motifs reduced the repressive activity of the gene. These results suggest that the hhole protein may interact with SH3-domain proteins or ERKs to mediate signaling pathways/networks that lead to the suppression of AP-1 and SRE.
© 2005 Elsevier Inc. All rights reserved.

Keywords: *hhole*; Six-transmembrane protein; SH3 domains; D-domain; Suppressor; MAPK signaling pathway

Mitogen-activated protein kinases (MAPKs) are major components of pathways controlling embryogenesis, cell differentiation, cell proliferation, and cell death. One of the most explored functions of MAPK signaling is the regulation of gene expression by direct or indirect phosphorylation and subsequent activation of transcription factors [1]. In mammals, MAP kinase superfamily is composed of several subfamilies including the extracellular signal-regulated kinase (ERK), JNK/SAPK, p38, and ERK5. p38 and JNK/SAPK are activated mainly by stress stimuli or inflammatory cytokines, whereas ERK is activated mainly by mitogenic stimuli [2,3]. Each MAPK has its own activators, substrates, and inactivators. Such a variety of molecules must transduce signals with high efficiency and specificity. What signals may be transmitted and received at various possible sites of action are determined by the spatial organization of kinases and substrates. There are two main mechanisms regulating the signal transduction in the MAP kinase cascades: the scaffolding and the docking interaction. Scaffolding generally requires a third molecule to tether enzymes and substrates [4]. The docking interaction is performed through protein–protein interactions which are mediated by specific conserved regions on MAPKs

☆ *Abbreviations:* TM, transmembrane; SH3, Src homology 3; MAPK, mitogen-activated protein kinase; MAPKK, MKK or MEK, MAPK kinase; MAPKKK or MEKK, a MAPKK kinase or MEK kinase; AP-1, activation protein 1; SRE, c-fos serum response element; DMEM, Dulbecco's modified Eagle's medium; DAPI, 4′,6′-diamidino-2-phenylindole hydrochloride.

* Corresponding authors. Fax: +86 0731 8615078.
E-mail addresses: xiushanwu@yahoo.com (X. Wu), mliu@ibt.tamhsc.edu (M. Liu).

[1] These authors contributed equally to the work.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.05.115

and MAPK-interacting molecules [5]. The MAPKs at the end of these signaling cascades phosphorylate their target proteins, many of which are nuclear proteins and many of which remain in the cytoplasm and other subcellular compartments, to regulate the expression of many genes in response to environmental stimuli [6].

Protein–protein interactions are principal events in cellular signal transduction. In a wide variety of intracellular proteins in living cells, the SH3 domain is known as one of the ubiquitous protein–protein interaction modules that contribute to constructing complicated signaling networks [7]. Mediated protein–protein interactions by SH3 domains are fulfilled by binding proline-rich sequence motifs in target proteins [8,9]. The SH3 domain comprised of approximately 60 residues is important for coupling of intracellular signaling pathways, regulation of catalytic activity of proteins, recruitment of substrates to enzymes, and localization of proteins to a specific subcellular compartment [10,11]. The SH3 ligand peptides can be divided into two distinct parts. The C-terminal part adopts a polyproline type II helix conformation, in which the core motif is XPpXP (where XP represents the SH3-domain binding dipeptides and p is a scaffolding residue, usually a proline) [12]. The two core prolines (XPpXP) so bind a well-conserved hydrophobic surface of the SH3 domain. And the N-terminal part binds to the SH3 domain in the region formed by the valley between the nSrc and RT-loops. In these proteins, proline-rich peptides only 10aa long serve as targets of SH3 binding [13]. And the SH3 domain contains a strip of approximately nine amino acid residues, which primarily recognize the proline-rich stretches [12]. It is clear that there is at least one highly conserved PXXP motif present in all SH3 ligands [14]. Proteins may bind some SH3-domain-containing proteins through the PXXP motifs, therefore activity cell signaling.

While searching for genes that are involved in heart development and diseases, we cloned a novel gene, *hhole*, from human embryonic heart cDNA library, *hhole* has a predicted 319-amino acid open reading frame, encoding a putative 35.8 kDa protein. hhole N-terminal region contains six transmembrane segments and ERK D-domain motif (a binding site of ERK), and its C-terminal contains proline-rich (PXXP) sequences, suggesting that hhole may have a potential role in cell signaling. Northern blot analysis demonstrated that *hhole* gene is expressed in heart, liver, pancreas, and skeletal muscle at adult stage and is expressed in most embryonic tissues, especially at a higher level in heart at both adult and embryo stages, suggesting that *hhole* gene might play a role in heart development. Labeled with green fluorescence protein showed that the hhole protein is localized to plasma membrane or proximal region of the membrane. Overexpression of hhole in COS-7 cells inhibits the transcriptional activities of AP-1 and SRE, in which the deletion of the C-terminal containing proline-rich site stretches or the N-terminal containing three D-domain motifs reduces the repressive activity of the gene. The results suggest that the hhole protein may function as a suppressor in MAPK signaling pathway.

Materials and methods

Construction of cDNA library of human embryonic heart. The 20-week human embryonic heart cDNA library was constructed as reported previously [15]. Briefly, 5 μg mRNA was purified from 500 μg total human embryonic heart RNA using Rapid mRNA purification Kit (Amresco). Reverse transcription reactions were performed with the purified embryonic heart mRNA and oligo(dT)-RA primer according to cDNA Synthesis kit protocol (TaKaRa). After Cassette Adaptor Ligation reactions using cDNA PCR Library Kit, cDNA amplification reactions were performed with RA primer, CA primer, and Ex Taq (TaKaRa).

Cloning and bioinformatics analysis. The amino acid sequence of chicken *hole* (AF488728) obtained from NCBI (http://www.ncbi.nlm.nih.gov) was used to search human EST database with the BLAST searching program (http://www.ncbi.nlm.nih.gov). Through combined BLAST search as previously described [16], a number of ESTs presenting the same gene human *hole* were identified in our database search. The first forward primer in BI548060 and the reverse primer in BE275761 (P1 and P2, Table 1), and the second forward primer in BG474572 and the reverse primer in BU071480 (P3 and P4, Table 1) were designed using Primer Premier 5.0 to perform standard PCR. Jellyfish 1.4 was used to find the open-reading frame (ORF) and the deduced translated product. Then, the coding sequence was cloned from human heart library with a pair of primers (PORF1 and PORF2, Table 1). All the PCR products were then cloned into pMD18T-vector (Sagon) and sequencing with 377 DNA Sequencer (ABI PRISM).

Primary sequence analysis for *hhole* was performed in BLAST (www.ncbi.nlm.nih.gov/BLAST/). Blastn program was used to identify the cytological locus of gene and to look for exons and introns. The sequence alignment and phylogenetic tree analysis were performed with MegAlign program (DNAstar). Secondary protein structure predictions were performed using the web tools SMART (smart.embl-heidelberg.de/), scansite (http://scansite.mit.edu/), CBS (http://www.cbs.dtu.dk/), and PSORT (psort.nibb.ac.jp/).

Plasmid construction. The following plasmids were constructed and used for mammalian cell transfections. To generate a fusion protein of hhole with green fluorescent protein (GFP), pEGFP-N1-hhole, the DNA fragment containing the hhole coding sequence, was amplified from pMD18T-hhole with primers EP1 (Table 1) containing *Xho*I restriction, and EP2 (Table 1) containing *Eco*RI restriction, and the amplified DNA fragment, cleaved with *Eco*RI/*Xho*I site, was ligated with pEGFP-N1 (Clontech), which had been cleaved with *Eco*RI/*Xho*I site.

pCMV-tag2-hhole (1–319) contains the full-length hhole protein, pCMV-tag2-PP (284–319) contains only the proline-rich region, and pCMV-tag2-DD (1–284) contains three ERK D-domain sites. pAS1

Table 1
The specific oligonucleotide sequences of primers

Primers	Sequences
P1	5′-CCATGCCCGCTGGCTGAGC-3′
P2	5′-CGCCCAGCTCAGTGATAGCC-3′
P3	5′-CCGTGTCTCGGCCATCTTCG-3′
P4	5′-ACTGCCCTCCCTTCCAACCC-3′
pORF1	5′-ATACTCGAGCGGCGGCCGACCATGGTG-3′
pOFR2	5′-TTTCTCGAG CTGTCACGTGTCCAGGGGGTCA-3′
EP1	5′-ATACTCGAGCGGCGGCCGACCATGGTG-3′
EP2	5′-AAAGAATTCCCGTGTCCAGGGGGTCACGC-3′

Download English Version:

https://daneshyari.com/en/article/10770045

Download Persian Version:

https://daneshyari.com/article/10770045

Daneshyari.com