Biochemical and Biophysical Research Communications 391 (2010) 698–702

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

FLJ23654 encodes a heart protein phosphatase 1-binding protein (Hepp1)

Chun-Yu Chen [a,1], Ning-Sheng Lai [b,1], Jaw-Ji Yang [c], Hsien-lu Huang [d], Wei-Chuan Hung [e], Chin Li [e], Ta-Hsien Lin [f,g,*], Hsien-bin Huang [e,*]

[a] Department of Chemistry and Biochemistry, National Chung Cheng University, Chia-Yi, Taiwan
[b] Section of Allergy, Immunology and Rheumatology, Department of Medicine, DaLin Tzu Chi Buddhist Hospital, Chia-Yi, Taiwan
[c] School of Dentistry, Chung-Sun Medical University, Taichung, Taiwan
[d] Department of Nutrition and Health Science, Fooyin University, Kaohsiung, Taiwan
[e] Department of Life Science and Institute of Molecular Biology, National Chung Cheng University, Chia-Yi, Taiwan
[f] Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan
[g] Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 3 November 2009
Available online 27 November 2009

Keywords:
Protein phosphatase 1
FLJ23654
Yeast two-hybrid screen
Inhibitor-1
Cardiac functions

ABSTRACT

In this report, we identified the novel protein heart protein phosphatase 1-binding protein (Hepp1), encoded by *FLJ23654*. Hepp1 associated with protein phosphatase 1 (PP1) by yeast two-hybrid, GST pull-down, co-immunoprecipitation, and far Western blotting assays. Northern blot analysis revealed that Hepp1 mRNA was only expressed in human heart and testis. Recombinant Hepp1 slightly enhanced the enzymatic activity of PP1 and antagonized the ability of phospho-inhibitor-1 or inhibitor-2 to inhibit PP1. Hepp1 protein in human heart tissues was detected by Western blot analysis. Together, our data suggest that Hepp1 can play a role in cardiac functions by working in concert with PP1.

Introduction

Protein phosphatase 1 (PP1), a major serine/threonine protein phosphatase, is associated *in vivo* with a family of regulatory subunits that target it to different subcellular locations and modulate its activity toward specific substrates [1–4] Most of the PP1 regulatory proteins contain a consensus PP1-binding motif, (R/K)(V/I)X(F/W), which binds to the surface of the enzyme distant from the active site [5–8]. More than 50 PP1 regulatory proteins have been identified [9], but several lines of evidence indicate that a number of novel PP1 regulatory proteins remain to be identified [10–12]. In a previous study, we employed the yeast two-hybrid method to identify several clones that encoded novel PP1 regulatory proteins [13]. One of them was phostensin, encoded by *KIAA1949*, which targets PP1 to the F-actin cytoskeleton. In the current study, we report that *FLJ23654* encodes a novel PP1-binding protein, which we have termed Hepp1 for heart protein phosphatase 1-binding protein. Hepp1 mRNA was exclusively expressed in heart and testis, and enhanced the activity of PP1, suggesting it may play an important role in cardiac function.

Materials and methods

Reagents. Tris, LB broth, DTT, ampicillin, phenylmethylsulfonyl fluoride, benzamidine and imidazole were obtained from Sigma. Thrombin, GSH-Sepharose, blue-Sepharose and metal chelating-Sepharose were obtained from Amersham Biosciences. PP1 and PP2A were prepared as described [14]. Phospho-inhibitor-1 and inhibitor-2 were prepared as described [15]. Proteins extracted from human heart tissues were obtained from Clontech.

5′-rapid amplification of cDNA ends (5′-RACE). 5′-RACE was performed using the BD Biosciences Marathon-Ready cDNA Kit (cat. No. 639304). Primers were designed following the protocol provided by the manufacturer. Touchdown PCR of human heart 5′ cDNA ends was performed using adaptor primer I provided by the manufacturer and a gene-specific primer (GGTGGGATGGGAGG TGTAATGTTTGGTG). The resulting product was used as template for nested PCR using the adaptor primer 2 provided by the manufacturer and a nested gene-specific primer (TCCCCTGTGGCTGG TAGAATTGGAACTG). The nested PCR product was isolated by agarose gel purification and ligated into pCR2.1-TOPO vector (Invitrogen). The cDNA insert was amplified, isolated and analyzed by DNA sequencing.

Northern blot analysis. Poly(A)$^+$ RNA from various human tissues (Clontech) was probed with the full-length Hepp1 cDNA labeled using random priming.

Co-immunoprecipitation. Rabbit PP1α cDNA was amplified by PCR using the primers (GCGCGAATTCAATGAGCCGCCTGTTC-

* Corresponding authors. Fax: +886 2 8751562 (T.-H. Lin); fax: +886 5 2722871 (H.-B. Huang).
E-mail addresses: thlin@vghtpe.gov.tw (T.-H. Lin), biohbh@ccu.edu.tw (H.-b. Huang).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.11.123

TATGGG) and (GCGCGGATCCTCACCGCCGGCAGGACTCATC). The resulting product was digested with EcoRI/BamHI and subcloned into pFLAG-CMV2 to make plasmid pPP1-FLAG. Full-length Hepp1 cDNA was subcloned from human heart cDNA by PCR using the primers GCGCGAATTCAATGAGCCGCCTGTTCTATGGG and GCGCGG ATCCTCACCGCCGGCAGGACTCATC. The amplified cDNA was digested with EcoRI/BamHI and subcloned into pEGFP-C1 to make plasmid pEGFP-Hepp1. Cultures of HEK293 cells were maintained at 5×10^5 cells/mL in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics (25 U/mL penicillin and 25 U/mL streptomycin) overnight. Cells were transfected at room temperature for 30 min using 5 μl of Lipofectamine 2000 and 2 μg of either pPP1-FLAG or pEGFP-Hepp1. Transfected cells were maintained at 37 °C for 2 days and lysed with 1% NP40 lysis buffer (20 mM Tris–HCl (pH 8.0), 1% NP40, 137 mM NaCl, 10% glycerol, sodium vanadate and protease inhibitors [1 mM phenylmethylsulfonyl fluoride, 0.2 U/mL aprotinin and 20 μg/mL leupeptin]). Cell extracts (300 μg) were incubated with 5 μl of anti-GFP Ab (Roche) for 60 min at 4 °C and mixed with 30 μl of protein A-Sepharose beads for an additional 60 min. Immunoprecipitates were collected by centrifugation, washed three times with the same buffer, and subjected to SDS–PAGE. Immunoblot analysis was performed using anti-FLAG mAb (Sigma).

GST pull-down assay. Full-length Hepp1 cDNA was amplified from pEGFP-Hepp1 by PCR using primers GCGCGAATTCATG GTCCTTCGGCCTCAGCA and GCGCGCGGCCGCATTCATTTGCTGGGAA ACACGGT. Hepp1 cDNA was cleaved with BamHI/EcoRI, ligated into $pGEX^{4T-1}$ (Amersham Biosciences), and expressed in BL21-DE3 *Escherichia coli*. The GST-Hepp1 fusion protein was purified by affinity chromatography on glutathione-Sepharose beads (Amersham Biosciences) using 10 mM glutathione/20 mM Tris–HCl (pH 7.5) for elution. After dialysis into buffer containing 20 mM Tris–HCl (pH 7.5), 0.2 mM EDTA, 0.2 mM DTT, 4 mM benzamidine, 0.02% NaN_3 and 150 mM NaCl to remove glutathione, 10 μg of the GST-fusion protein was incubated with 2 μg of recombinant PP1α in 300 μl of buffer (20 mM Tris–HCl (pH 7.5), 0.2 mM EDTA, 0.02% sodium azide, 2 mM DTT, 4 mM benzamidine and 0.1% NP40) for 60 min at 4 °C, followed by addition of 50 μl of 50% glutathione-agarose beads to precipitate the GST-fusion protein complex. The precipitates were analyzed by SDS–PAGE and were subjected to Western blot using anti-PP1α Ab.

Site-directed mutagenesis. GST-Hepp1 (V100G), GST-Hepp1 (F102G) and GST-Hepp1 (V100G/F102G) were mutated using the QuikChange Site-directed Mutagenesis Kit (Stratagene) with appropriate primers following the methods described by the manufacturer.

Protein preparation. Full-length Hepp1 cDNA was cloned from a human heart cDNA library and was amplified by PCR using the primers (GCGCCCATGGGCCTGGTGCCACGCGGTTCTATGGTCCTTCG GCCTCAG) and (GCGCGAATTCTCATTTGCTGGGAAACACGGTCTG). The resulting product was digested with NcoI/EcoRI and subcloned into pET32a. *E. coli* BL21(DE3) was transformed with recombinant pET32a plasmid coding for thioredoxin-Hepp1(trx-Hepp1) with a thrombin cutting site upstream of Hepp1. Bacteria were grown in 1 L of LB broth containing ampicillin (0.1 g/L) at 37 °C with shaking at 250 rpm. Cultures were induced with 1 mM IPTG for 4 h once

```
   1 agcactgcttacccagccccacccaacctgctgccagccgcccctgctgctggtctctct
  61 gtcctataattccccagatggatccctgcagagacccctccagtccgggatccccactct
                     M  D  P  C  R  D  P  S  S  P  G  S  P  L  S
 121 cgtggtaggctccctcagacgcagcccaccatggtccttcggcctcagcagttccaatt
       W  *                           M  V  L  R  P  Q  Q  F  Q  F   10
 181 ctaccagccacaggggatcccctcctccccctcagccgtggtggtggagatggggtccaa
      Y  Q  P  Q  G  I  P  S  S  P  S  A  V  V  V  E  M  G  S  K   30
 241 gcctgccctcacgggggagcccgccctcacgtgcatcagcaggggcagtgaggcccggat
      P  A  L  T  G  E  P  A  L  T  C  I  S  R  G  S  E  A  R  I   50
 301 ccactccgcggccagctccctcattatggaagacaaagaaatccccatcaagagtgagcc
      H  S  A  A  S  S  L  I  M  E  D  K  E  I  P  I  K  S  E  P   70
 361 tctgccaaaaccgcccgcatctgccccaccatccatcctggtgaaaccagaaaactcaag
      L  P  K  P  P  A  S  A  P  P  S  I  L  V  K  P  E  N  S  R   90
 421 aaatggcatcgaaaagcaagtcaaaaccgtgagatttcagaattacagccctcctcccac
      N  G  I  E  K  Q  V  K  T  V  R  F  Q  N  Y  S  P  P  P  T  110
 481 caaacattacacctcccatcccacctccggaaagcctgaacagccagccaccctcaaggc
      K  H  Y  T  S  H  P  T  S  G  K  P  E  Q  P  A  T  L  K  A  130
 541 gtcccagcctgaagcagcgtccttgggcccagagatgaccgtcctatttgcccaccgaag
      S  Q  P  E  A  A  S  L  G  P  E  M  T  V  L  F  A  H  R  S  150
 601 tggctgccactccggacagcagacagacctccggagaaagtcagctcttggcaaggccac
      G  C  H  S  G  Q  Q  T  D  L  R  R  K  S  A  L  G  K  A  T  170
 661 aaccctggtgtccactgcctcaggcacgcagaccgtgtttcccagcaaatgaacctacgg
      T  L  V  S  T  A  S  G  T  Q  T  V  F  P  S  K  *            186
 721 gtggcttttcctagaccccaaagaggtgaattgcatttaaatacagtctgcctccactga
 781 gggcatcctgccattctttggggacttgagcatgggtccttgttcttcctatttcacctc
 841 caggaaagcaaaagtgggagcagaaattcctgccctgggtgggaggatagatggcgtggc
 901 cttccaaacatacaaacataatgatttgatgccacaaagctcacttactcagaccaagga
 961 gtgaaaattgtcgtgcccacttttatgccccagcatgagtatgtgactctgtcatctcgtg
1021 tactgtgtagatttctatgtgtctaaggggctgcagcagggtgtgacacgttggatggtg
1081 gcgtgctcgtgactacaaatcttacaggcttgaaaaggagaaaatctctcgagagactcc
1141 ttgaattc
```

Fig. 1. cDNA and protein sequence of human Hepp1. Nucleotides are numbered on the left and amino acids are numbered on the right. ATG and stop codons are boxed. The PP1-binding motif is underlined.

absorbance at 600 nm was between 0.6 and 1.0. Cells were harvested by centrifugation at 6000*g* for 10 min and the resulting pellet was suspended in 100 mL of buffer containing 20 mM Tris–HCl (pH 7.9), 0.5 M sodium chloride, 0.2 mM phenylmethylsulfonyl fluoride, 0.02% sodium azide and 5 mM imidazole, followed by two passes through a French Press at 1000–1500 psi. Insoluble material was removed by centrifugation at 20,000*g* for 20 min, and the supernatant was loaded onto a Ni-Sepharose column (2.5 × 10 cm). After washing with one volume of the same buffer, the column was developed with a linear imidazole gradient from 0.5 mM (500 mL) to 250 mM (500 mL) in the same buffer. The fractions containing trx-Hepp1 were pooled and concentrated to 5 mL by ultrafiltration using a YM-10 membrane. All components were diluted with 45 mL of buffer containing 20 mM Tris–HCl buffer (pH 7.5), 0.3 mM DTT, 0.02% sodium azide, and 0.2 mM EDTA, and were purified on a blue-Sepharose column that was developed with a linear gradient from 0.0 M (500 mL) to 1.0 M (500 mL) sodium chloride in the same buffer. Fractions containing trx-Hepp1 were pooled and concentrated by ultrafiltration, and were dialyzed against thrombin cutting buffer (20 mM Tris–HCl, pH 7.5). The dialyzed product was concentrated to 1 mg/mL and was cleaved with thrombin (2.5 mg of trx-Hepp1:1 μg of thrombin) for 2.5 h. The sample was further purified by FPLC using the Mono-S column (10 × 1 cm) with a linear gradient of 0.0–0.5 M NaCl between in buffer containing MOPS (pH 7.0), 1 mM DTT, 0.2 mM EDTA and 0.02% NaN_3. Fractions containing Hepp1 were pooled, dialyzed against 50 mM Tris–HCl (pH 7.0), and stored at 4 °C.

Yeast two-hybrid and protein phosphatase activity assays. The yeast two-hybrid screen was carried out as described [13]. AH109 yeast was transformed with the vector encoded the GAL-4 DNA-binding domain fused with rabbit PP1α. The transformed AH109 yeast was mated with Y187 yeast containing a pre-transformed MATCHMAKER human heart cDNA library. PP1 and PP2A activity assays were performed according to previously described methods [14].

Results and discussion

Identification of PP1 regulatory proteins

We conducted a search for proteins capable of interacting with PP1 in human heart using a yeast two-hybrid screen. Eight distinct classes of cDNAs were obtained from 39 positive clones. Twenty-two positive clones were from one unique class, suggesting that the heart contains an abundance of this class of cDNAs. DNA sequencing analysis indicated that these 22 clones were the product of the gene *FLJ23654*. We then used 5′-RACE analysis to determine the 5′ sequence of the *FLJ23654* transcript (GenBank Accession Number FJ695509), and we identified two AUG codons at nucleotides 78 and 152. If translation begins from the first AUG start codon, then the product is a small peptide containing 16 amino acids. However, this translated peptide lacks the PP1-binding motif and it is presumably unable to bind PP1. The second AUG codon is within Kozak context, and translation from this site

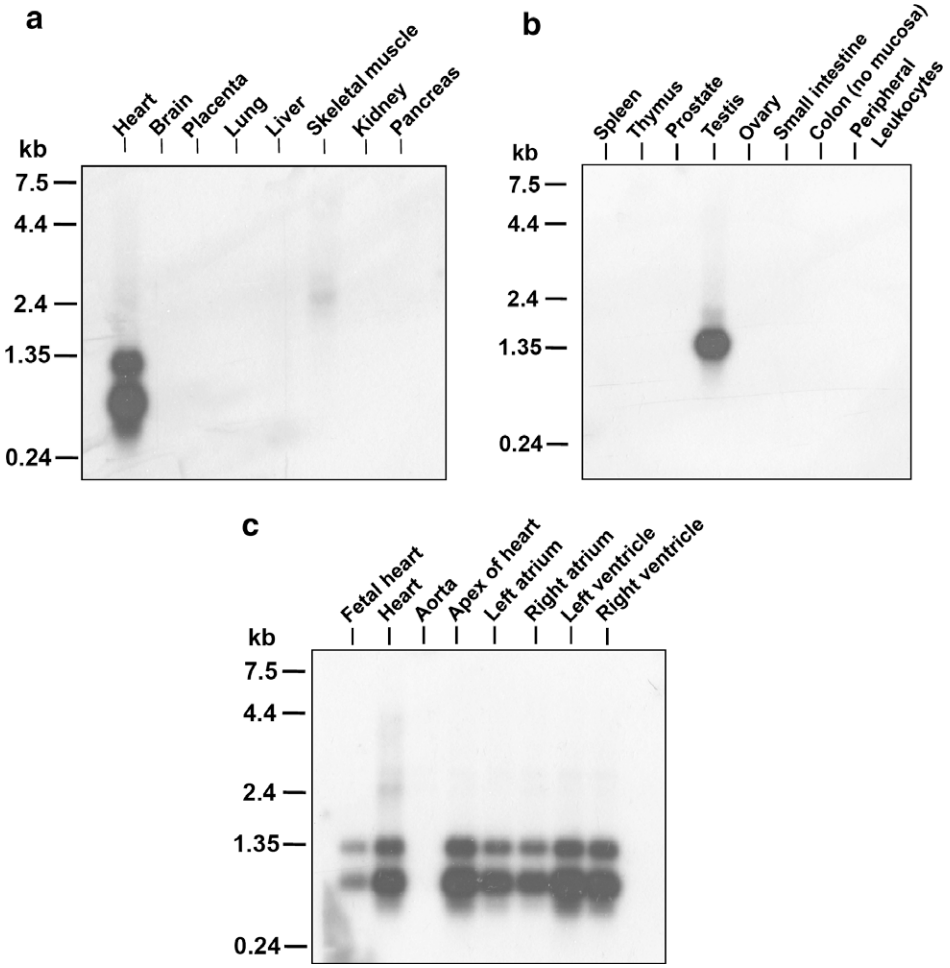

Fig. 2. Expression pattern of Hepp1. Hepp1 mRNA expression levels were detected by Northern blot analysis using Hepp1 cDNA as a probe.

results in a product consisting of 186 amino acids with a consensus PP1-binding motif located between residues 98–102 (Fig. 1). We named the product of the *FLJ23654* transcript the heart protein phosphatase 1-binding protein, or Hepp1.

PP1 interacts with Hepp1 by GST pull-down and co-immunopreci pitation assays

We confirmed the PP1–Hepp1 interaction by GST pull-down assay. The GST-Hepp1 fusion protein was prepared and incubated with PP1α. The GST-Hepp1:PP1 complex was co-precipitated using GSH-Sepharose (Fig. S1A). Site-directed mutagenesis was employed to mutate the PP1-binding motif of Hepp1. A single mutation (valine-100 replaced by glycine or phenylalanine-102 by glycine) or a double mutation (valine-100/phenylalanine-102 substituted by glycine/glycine) in Hepp1 significantly disrupted PP1 binding (Fig. S1A). Hepp1 binding to PP1 was also confirmed by co-immunoprecipitation using EGFP-Hepp1 and FLAG-PP1 (Fig. S1B). In this experiment, pFLAG-PP1 and pEGFP-Hepp1 plasmids were transiently co-expressed in HEK293 cells. FLAG-PP1 co-immunoprecipitated with EGFP-Hepp1, suggesting that Hepp1 forms a complex with PP1α Mutations of the PP1-binding motif of Hepp1 completely disrupted PP1 binding (Fig. S1B). Interaction between Hepp1 and PP1 was also confirmed by far Western blot (data not shown).

Hepp1 mRNA is expressed exclusively in heart and testes

Northern blot analysis revealed the presence of two major Hepp1 mRNA transcripts, approximately 1.24 and 0.68 kilobases in size, exclusively in heart and testis (Fig. 2A and B). This unusual expression pattern allowed us to further analyze the distribution of Hepp1 mRNA in human heart. Fig. 2C shows that Hepp1 mRNA transcripts are present in the apex, left atrium, right atrium, left ventricle and right ventricle, but not in the aorta.

Recombinant Hepp1 enhances the activity of PP1

We prepared a recombinant trx-Hepp1 fusion protein with a $(His)_6$ tag and a thrombin cutting site upstream of the Hepp1 sequence. Recombinant trx-Hepp1 was purified by Ni^{2+}-Sepharose chromatography and then by blue-Sepharose chromatography. Trx was cleaved from trx-Hepp1 by thrombin and Hepp1 was purified by FPLC using a Mono-S column. SDS–PAGE analysis followed by Coomassie Blue staining indicated that a single major band was present in the sample (Fig. 3A). The homogeneity of recombinant Hepp1 was greater than 95%, and its apparent molecular weight was approximately 25 kDa, which was more than the theoretical molecular weight of 19.88 kDa. We next assessed the functionality of recombinant Hepp1 by including it in PP1 inhibition assays. The catalytic activity of PP1 purified from rabbit muscle was not inhibited by 10 μM Hepp1, but was slightly increased to 117 ± 6%. However, in the presence of 100 or 10 nM of inhibitor-2, the activity of PP1 was reduced to 17 ± 3% and 45 ± 4%, respectively (Fig. 3B). Hepp1 can slightly enhance the activity of PP1 and antagonized the inhibition of PP1 by phospho-inhibitor-1 and by inhibitor-2. In the absence of Hepp1, the IC_{50}s for the inhibition of PP1 by phosphorinhibitor-1 and inhibitor-2 were 1.5 ± 0.2 and 3.4 ± 0.8 nM, respectively (Fig. 3C). In the presence of 0.1 μM Hepp1, the IC_{50}s were increased to 21 ± 3 and 32 ± 3 nM, respectively.

Hepp1 is present in human heart

Hepp1 protein is a product that is translated from the second AUG codon of its mRNA. We analyzed whether Hepp1 protein is present in human heart tissue. An aliquot of proteins extracted

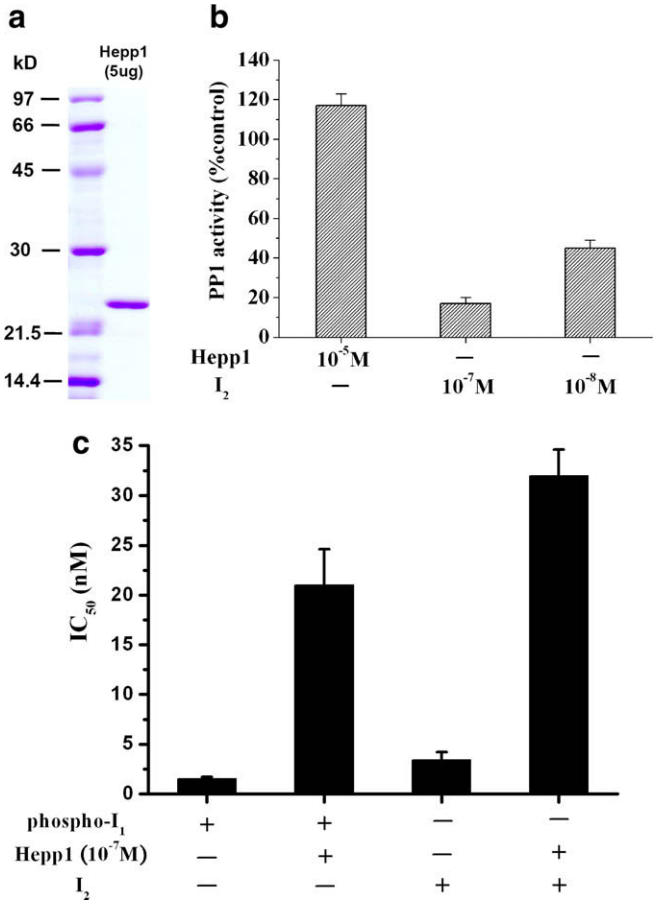

Fig. 3. Modulation of PP1 catalytic activity by Hepp1. (A) SDS–PAGE (12.5%) analysis of recombinant Hepp1 (5 μg). (B) Effects of Hepp1 and inhibitor-2 (I_2) on the catalytic activity of PP1. PP1 activity (mean ± S.D., $n = 3$) is expressed as the percent of activity measured in the absence or presence of Hepp1 or inhibitor-2. (C) Hepp1 antagonizes the inhibition of PP1 by phospho-inhibitor-1 ($p\text{-}I_1$) and inhibitor-2. The IC_{50} represents that 50 percents of PP1 activity are inhibited by the inhibitors (mean ± S.D., $n = 3$). In experiments, PP1 is assayed using 10 μM [^{32}P] phosphorylase a as substrate.

from human heart tissue was analyzed by Western blot, using anti-Hepp1 polyclonal antibodies. Fig. 4 shows that Hepp1 protein is present in human heart tissue and its mobility on SDS–PAGE is identical to that of the recombinant Hepp1 marker. The detected band disappeared upon pre-neutralization of the antibody with the recombinant Hepp1.

In conclusion, we have identified a novel PP1 regulatory protein, Hepp1, by a yeast two-hybrid screen. Hepp1 is encoded by *FLJ23654*, and northern blot analysis indicated that Hepp1 mRNA was only present in human heart and testis tissues. 5′-RACE analysis identified two AUG codons within the sequence. Translation can be initiated at the second AUG to result in a protein that contains 186 amino acids in length with a PP1-binding motif between residues 98 and 102. Hepp1 is present in human heart tissue. Interaction between Hepp1 and PP1 was observed by GST pull-down, co-immunoprecipitation, and far Western blot assays. Hepp1 slightly enhanced the activity of PP1, and antagonized the activity of phospho-inhibitor-1 or inhibitor-2 in its ability to inhibit PP1.

Several lines of evidence suggest that PP1 plays a critical role in cardiac function [16–19]. PP1 is a negative regulator, in that overactivation of PP1 or reduction of inhibitor-1 and inhibitor-2 activity was observed in failing heart tissues. Hepp1 can slightly enhance the activity of PP1, and antagonizes the activities of phospho-inhibitor-1 and inhibitor-2, suggesting that Hepp1 can affect

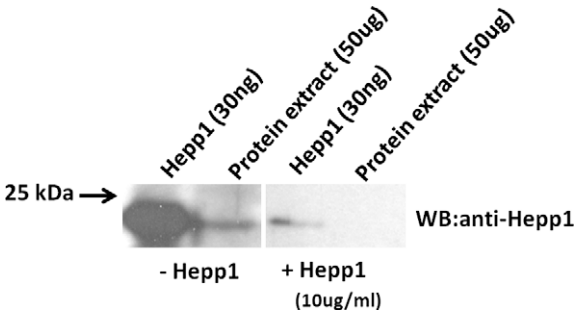

Fig. 4. Hepp1 present in human heart tissues. An aliquot of proteins (50 μg) extracted from the human heart is analyzed by Western blot using anti-Hepp1 polyclonal antibodies. An immunoreactive protein migrating at ~25 kDa is identified and shows an identical migration on SDS–PAGE with the recombinant Hepp1 (30 ng). Pre-incubation of the recombinant Hepp1 with anti-Hepp1 before immunoblotting is able to neutralize the recognition.

cardiac function through modulation of PP1. In addition, Hepp1 mRNA was only present in human testis and heart tissues in which of the regions include the apex, left atrium, right atrium, left ventricle and right ventricle, but not in the aorta. Therefore, Hepp1 protein can be a potential novel biomarker for diagnosis of cardiac injury.

Acknowledgments

This work was supported by grants from the National Science Council, ROC (NSC 94-2320-B-194-005 and NSC 95-2320-B-194-005) and the Taipei Veterans General Hospital, Taiwan, ROC (VGH 92-375, VGH 93-359 and VGH 94-370).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.11.123.

References

[1] P. Cohen, The structure and regulation of protein phosphatases, Annu. Rev. Biochem. 58 (1989) 453–508.
[2] S. Shenolikar, A.C. Nairn, Protein phosphatases: recent progress, Adv. Second Messenger Phosphoprotein Res. 23 (1991) 1–121.
[3] M.J. Hubbard, P. Cohen, On target with a new mechanism for the regulation of protein phosphorylation, Trends Biochem. Sci. 18 (1993) 172–177.
[4] S. Shenolikar, Protein serine/threonine phosphatases – new avenues for cell regulation, Annu. Rev. Cell Biol. 10 (1994) 55–86.
[5] M.P. Egloff, D.F. Johnson, G. Moorhead, P.T.W. Cohen, P. Cohen, D. Barford, Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1, EMBO J. 16 (1997) 1876–1887.
[6] S. Zhao, E.Y.C. Lee, A protein phosphatase-1-binding motif identified by the panning of a random peptide display library, J. Biol. Chem. 272 (1997) 28368–28372.
[7] Y.G. Kwon, H.B. Huang, F. Desdouits, J.A. Girault, P. Greengard, A.C. Nairn, Characterization of the interaction between DARPP-32 and protein phosphatase 1 (PP-1): DARPP-32 peptides antagonize the interaction of PP-1 with binding proteins, Proc. Natl. Acad. Sci. USA 94 (1997) 3536–3541.
[8] J.H. Connor, H.N. Quan, N.T. Ramasawamy, L. Zhang, S. Barik, J. Zheng, J.F. Cannon, E.Y.C. Lee, S. Shenolikar, Inhibitor-1 interaction domain that mediates the inhibition of protein phosphatase-1, J. Biol. Chem. 273 (1998) 27716–27724.
[9] P.T.W. Cohen, Protein phosphatase 1-targeted in many directions, J. Cell Sci. 115 (2002) 241–256.
[10] M. Campos, P. Fadden, G. Alms, Z. Qian, T.A. Haystead, Identification of protein phosphatase-1-binding proteins by microcystin-biotin affinity chromatography, J. Biol. Chem. 271 (1996) 28478–28484.
[11] G.B. Moorhead, C. MacKintosh, N. Morrice, P. Cohen, Purification of the hepatic glycogen-associated form of protein phosphatase-1 by microcystin-Sepharose affinity chromatography, FEBS Lett. 362 (1995) 101–105.
[12] H.T. Tran, A. Ulke, N. Morrice, C.J. Johannes, G.B. Moorhead, Proteomic characterization of protein phosphatase complexes of the mammalian nucleus, Mol. Cell. Proteomics 3 (2004) 257–265.
[13] S.C. Kao, C.Y. Chen, S.L. Wang, J.J. Yang, W.C. Hung, Y.C. Chen, N.S. Lai, H.T. Liu, H.L. Huang, H.C. Chen, T.H. Lin, H.B. Huang, Identification of phostensin, a PP1 F-actin cytoskeleton targeting subunit, Biochem. Biophys. Res. Commun. 356 (2007) 594–598.
[14] P. Cohen, S. Alemany, B.A. Hemmings, T.J. Resink, P. Stralfors, H.Y. Lim Tung, Protein phosphatase-1 and protein phosphatase-2A from rabbit skeletal muscle, Methods Enzymol. 159 (1988) 390–408.
[15] H.B. Huang, Y.C. Chen, T.T. Lee, Y.C. Huang, H.T. Liu, C.K. Liu, H.J. Tsay, T.H. Lin, Structural, biochemical characterization of inhibitor-1$_\alpha$, Proteins 68 (2007) 779–788.
[16] A.N. Carr, A.G. Schmidt, Y. Suzuki, F. del Monte, Y. Sato, C. Lanner, K. Breeden, S.L. Jing, P.B. Allen, P. Greengard, A. Yatani, B.D. Hoit, I.L. Grupp, R.J. Hajjar, A.A. DePaoli-Roach, E.G. Kranias, Type 1 phosphatase, a negative regulator of cardiac function, Mol. Cell. Biol. 22 (2002) 4124–4135.
[17] R.C. Gupta, S. Mishra, S. Rastogi, M. Imai, O. Habib, H.N. Sabbah, Cardiac. SR-coupled, PP1 activity and expression are increased and inhibitor 1 protein expression is decreased in failing hearts, Am. J. Physiol. Heart Circ. Physiol. 285 (2003) H2373–H2381.
[18] A. El-Aromuche, T. Pamminger, D. Ditz, O. Zolk, T. Eschenhagen, Decreased protein and phosphorylation level of the protein phosphatase inhibitor-1 in failing human hearts, Cardiovasc. Res. 61 (2004) 87–93.
[19] R.C. Gupta, S. Mishra, X.P. Yang, H.N. Sabbah, Reduced inhibitor 1 and 2 activity is associated with increased protein phosphatase type 1 activity in left ventricular myocardium of one-kidney, one-clip hypertensive rats, Mol. Cell. Biochem. 269 (2005) 49–57.