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Molecular cloning and characterization of porcine cDNA encoding a 90-kDa heat shock protein and its expression following hyperthermia

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Abstract

We have isolated and sequenced cDNA clones encoding a 90-kDa heat shock protein (HSP90) from a porcine brain cDNA library. The sequence of the 2202-nucleotide coding region showed 88.6% homology with that of the human homologue. Moreover, the deduced amino acid sequence of the porcine *hsp90* cDNA was 99.7% identical to that of the human counterpart, with a difference of only three amino acids in a total of 733 residues. Expression of the gene was greatly increased in cultured cells during recovery from heat shock treatment at 45°C for 60 min. Three major transcripts 2.2, 3.0, and 4.1 kb in size were detected by Northern blot hybridization. These transcripts were further identified in a whole-pig hyperthermia experiment. These three *hsp90* transcripts were constitutively expressed in porcine tissues including kidney, liver, brain, and heart, and their levels were markedly enhanced during recovery from 30-min hyperthermia treatment at 43°C. Furthermore, we found that HSP90 was preferentially expressed in pituitary gland, brain, adrenal gland, and testis, in comparison to the other tissues.

Keywords: Nucleotide; RT-PCR; Northern hybridization; Western blotting

1. Introduction

Living organisms are capable of producing proteins in response to environmental changes such as temperature elevation (Lindquist and Craig, 1988), exposure to oxidative stress (Liao et al., 1994), and myocardial ischemia (Benjamin and Williams, 1994; Mestril and Dillmann, 1995). These proteins are highly conserved among various organisms and collectively termed heat shock proteins (HSPs). According to their apparent molecular weights and degrees of homology, HSPs are classified into several families. The major classes of mammalian HSPs are HSP90s (83–99 kDa), HSP70s (68–80 kDa), HSP60s, and the small HSPs (25–28 kDa) (Lindquist and Craig, 1988).

There is circumstantial evidence showing that HSP70 functions as a molecular chaperone (Hendrick and Hartl, 1993; James et al., 1997). Similar to HSP70, the HSP90 is also a molecular chaperone, which is conserved among all living organisms to protect cells against stress (Jakob and Buchner, 1994; Nathan et al., 1997). It is believed that HSP90 possesses the ability to refold denatured proteins into proper conformations (Wiech et al., 1992; Jakob and Buchner, 1994). In fact, HSP90 associates with steroid hormone receptors and maintains them in a non-functional state until hormone binding (Smith and Toft, 1993; Czar et al., 1997). Furthermore, HSP90 also interacts with other nuclear or cytoplasmic proteins, including transforming or regulatory tyrosine kinases (Aligue et al., 1994), some serine/threonine kinases (Stancato et al., 1993), transcription factors (Sanchez et al., 1985), cytoskeletal proteins (Koyasu et al., 1986; Czar et al., 1996), calmodulin (Minami et al., 1991), and $\beta\gamma$ subunits of G proteins (Inanobe et al., 1994).

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Abbreviations: HCM, hypertrophic cardiomyopathy; HSP90, 90-kDa heat shock protein; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecylsulfate; SMC, smooth muscle cells; SPF, specific-pathogen free.

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In a previous study (Lee et al., 1996), we found that the expression of HSP90 in cardiac tissue was substantially reduced in sudden-death pigs with hypertrophic cardiomyopathy (HCM). This was the first evidence correlating a reduced HSP90 level in ventricular tissue with cardiac arrest in HCM pigs. To explore the molecular mechanism(s) of HSP90 in the pathogenesis of cardiovascular disease, we cloned porcine *hsp90* cDNA and determined its heat inducibility.

2. Materials and methods

2.1. Library screening and sequencing protocol

A probe was generated to screen a porcine brain cDNA library in phage λgt11 (Clontech Lab., California, USA) with primers corresponding to nucleotides 929–1522 of the human *hsp90α* cDNA sequences (Fig. 1). This region is highly conserved between human and mouse genomes (Moore et al., 1989; Yamazaki et al., 1989). A 594-bp PCR product was obtained using porcine heart cDNA as a template, and its nucleotide sequence was confirmed. The library was plated out on twenty 150-mm plates at a density of 2×10^4 plaques per plate. Hybridization of cDNA clones with the probe, isolation and amplification of recombinant phage DNA, and DNA sequencing were all carried out according to standard procedures (Sambrook et al., 1989). DNA sequences determined manually were consistent with those from an automatic DNA sequencer (ABI 377, Perkin-Elmer, California, USA).

2.2. Heat treatment of smooth muscle cells and pigs

Smooth muscle cells (SMC) were isolated from porcine aorta according to the procedure described by Chamley-Campbell et al. (1979). The SMC between passages 8 and 12 were cultured in 75 cm^2 flasks containing Dulbecco's modified Eagle's medium supplemented with 10% FBS at a 5% CO_2 and 37°C incubator. When the cells reached sub-confluence, the cultures were heated at 45°C for 60 min and then recovered at 37°C for 0–8 h.

Specific-pathogen free (SPF) pigs (20–30 kg) were pre-anesthetized with an intramuscular injection of zaperonum (2 mg/kg) and atropine (0.05 mg/kg). The animals were then anesthetized with thiamylal sodium (10 mg/kg) intravenously and were subjected to hyperthermia by exposure to a heating pad set at 55°C until their rectal temperature reached 43°C and remained constant for 30 min. Control SPF pigs were kept on a surgical table without exposure to the heating pad. Their rectal temperature was also recorded. The animals were quarantined in their normal environment for 24 h after hyperthermia treatment and were subsequently sacrificed for organ harvest (heart, kidney, liver, etc.). The tissues were removed and frozen immediately in liquid nitrogen.

2.3. RNA extraction and RT-PCR analysis

Total RNA was isolated using the RNeasy Mini kit (Qiagene, Hilden, Germany) according to the manufacturer's instructions. The cDNA was synthesized by first heating 12 μl of reactive solution containing 5 μg of total RNA and 10 μM oligo $(dT)_{16}$ primer at 75°C for

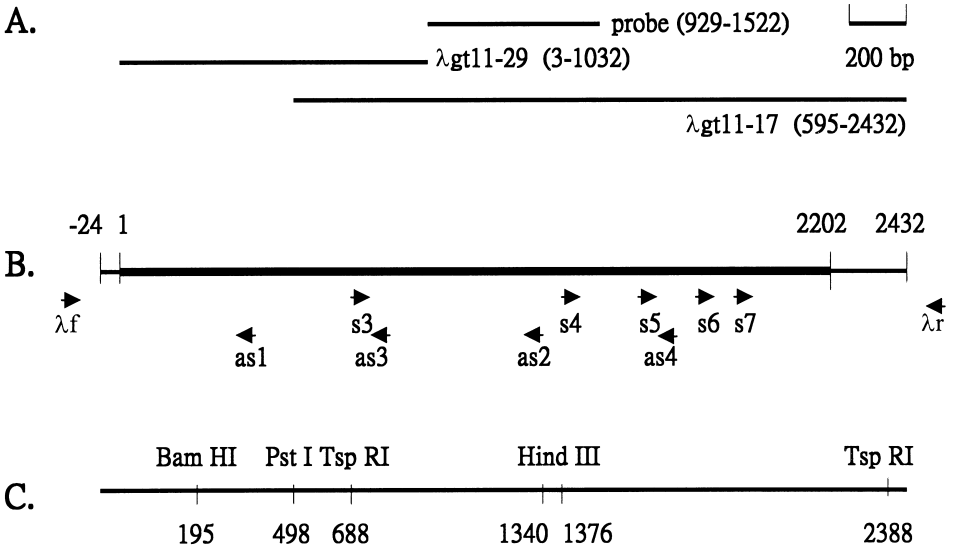

Fig. 1. Sequencing strategy and restriction map of porcine *hsp90* cDNA. (**A**) Two clones isolated from a porcine brain cDNA library using a probe (dashed line). The cDNAs were PCR cycle-sequenced by autosequencer. (**B**) Locations and directions of the forward primers (s3–s7), λf, λr, and reverse primers (as1–as4). Nucleotide sequence −24 to 2 was obtained from sequencing a cDNA fragment in testis cDNA library unidirectionally. The restrictioned sites in (**C**) were confirmed by enzyme digestion. Numbers shown below indicate the location in the complete cDNA sequence.

15 min, followed by adding 20.5 units of reverse transcriptase (SuperRT, HT Biotechnology Cambridge, UK), 40 units of ribonuclease inhibitor (HT Biotechnology), 1 mM of mixed deoxyribonucleotide triphosphate, 1 × RT buffer (50 mM Tris–HCl, pH 8.3; 50 mM KCl; 4 mM dithiothreitol; 10 mM $MgCl_2$), and diethylpyrocarbonate (DEPC)-treated water to a total volume of 20 μl. The mixture was incubated at 37°C for 1 h and chilled on ice for 5 min, and the reaction was terminated by heating at 75°C for 10 min, and stored at −20°C.

The multiplex PCR reaction mixture of 25 μl contained 1 μl of cDNA, 1 × PCR reaction buffer [50 mM KCl; 10 mM Tris–HCl, pH 8.3; 1.5 mM $MgCl_2$; and 0.01% (w/v) gelatin], 2 μM of the primer pairs, and 0.5 units of DNA polymerase (Super Taq, HT Biotechnology). The following primers were used: forward 5′-AGGAGAACCAGAAACACATC-3′ and reverse 5′-AAGCCAGAAGACAGCAGAGC-3′ were designed in accordance with porcine *hsp90* cDNA sequence (see also Fig. 2); forward 5′-GCGGGAC-ATCAAGGAGAA-3′; reverse 5′-GAAGGAGGGCTG-GAAGAG-3′ for porcine *β*-actin gene (GenBank Accession No. U07786) as a control. PCR was carried out for 30 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C in a DNA Thermal Cycler (Perkin-Elmer 9600, Norwalk, CT). Ten microliters of PCR products were electrophoresed in a 2% agarose gel followed by ethidium bromide staining and photographed using a 667 Polaroid camera (Polaroid, Hertfordshire, UK).

2.4. Northern blot analysis

Twenty micrograms of total RNA were denatured, electrophoresed on 1% agarose gel in the presence of formaldehyde, and transferred to a positive charge membrane (Ambion, Texas, USA) by a capillary method. After cross-linking, the filter was prehybridized for 2 h at 65°C, followed by an incubation with the biotinylated riboprobe at the same temperature overnight in the hybridization buffer. Following hybridization, the blot was washed three times in a wash solution. All of the reagents used and procedures described above are included in the NorthernMax kit (Ambion). To prepare the biotinylated riboprobe, the instructions described in a non-isotopic in-vitro transcription kit (Ambion) were followed. A 1159-bp PCR product of *hsp90* (nucleotide 872–2100, Fig. 2) was used as the template, and 21 nucleotides of the T7 promoter were included at the 5′ end of the reverse primer with the sequence 5′-TAATACGACTCACTATAGGGAAGCCAGAAG-ACAGCAGAGC-3′ and the forward primer 5′-TCAACAAGACAAAGCCTATC-3′. The hybridization signals were detected by a non-isotopic detection kit (Ambion) as described in the manufacturer's protocols.

2.5. Western blot analysis

Western blot analysis and tissue homogenization were conducted, as described previously (Lee et al., 1996). The crude homogenates were centrifuged at 12 000 rpm for 5 min at 4°C. The supernatant was lysed in sample buffer (62.5 mM Tris–HCl, pH 6.8; 2% SDS; 5% *β*-mercaptoethanol; 10% glycerol; and 0.002% Bromophenol Blue), heated in boiling water for 5 min, and then microfuged at full speed for 3 min. Protein concentration was determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as a standard. One hundred micrograms of the samples were used for 9% SDS–polyacrylamide gel electrophoresis (Laemmli, 1970) and transferred to a nitrocellulose membrane (Hybond-C extra, Amersham) by a semi-dry method (OWL Scientific Plastics, Cambridge, UK). The blot was incubated for 1 h with 3% gelatin in TTBS (20 mM Tris–HCl, pH 7.4; 500 mM NaCl; 0.05% Tween 20) and then rinsed with TTBS. Subsequently, a polyclonal antibody against porcine HSP90 (diluted 1:500; to be published elsewhere) was added and incubated for 1 h at room temperature. After three washes with TTBS, the membrane was incubated with goat anti-rabbit antibody conjugated with alkaline phosphatase (Sigma; diluted 1:2000 in TTBS containing 1% gelatin) at room temperature for 30 min. The membrane was then rinsed three times with TTBS and developed at room temperature in a developing buffer (15 mg of Nitro Blue Tetrazolium; 0.7% *N*,*N*-dimethylformamide; 30 mg of 5-bromo-4-chloro-3-indolyl phosphate per 100 ml; 1 mM $MgCl_2$; and 100 mM $NaHCO_3$, pH 9.8).

3. Results

3.1. Isolation and analysis of porcine hsp90 cDNA sequences

A porcine brain cDNA library in λgt11 vector was screened with a 594-bp PCR product (Fig. 1) containing a high-homology region of mouse and human *hsp90α*. Among the 20 positive clones, two were 1.03 and 1.4 kb in length and were located at the 5′ end (denoted λgt11–29), and the 3′ end (denoted λgt11–17), respectively. DNA sequencing of the two cDNAs revealed that the complete porcine *hsp90* cDNA sequence consisted of 24 bp of 5′-untranslated region (Fig. 2), a 2202-bp open reading frame, and 230 bp of the 3′-untranslated region. The sequence upstream of the third nucleotide G of the start codon was derived from cDNA fragments in a porcine testis cDNA library. The open reading frame predicts a polypeptide of 733 amino acids.

The coding sequence of porcine *hsp90* and the deduced amino acid sequence (Figs. 2 and 3) shared 88.6 and 99.7% identity with those of the human hsp90 sequences (Yamazaki et al., 1989; Fig. 3), respectively. There were

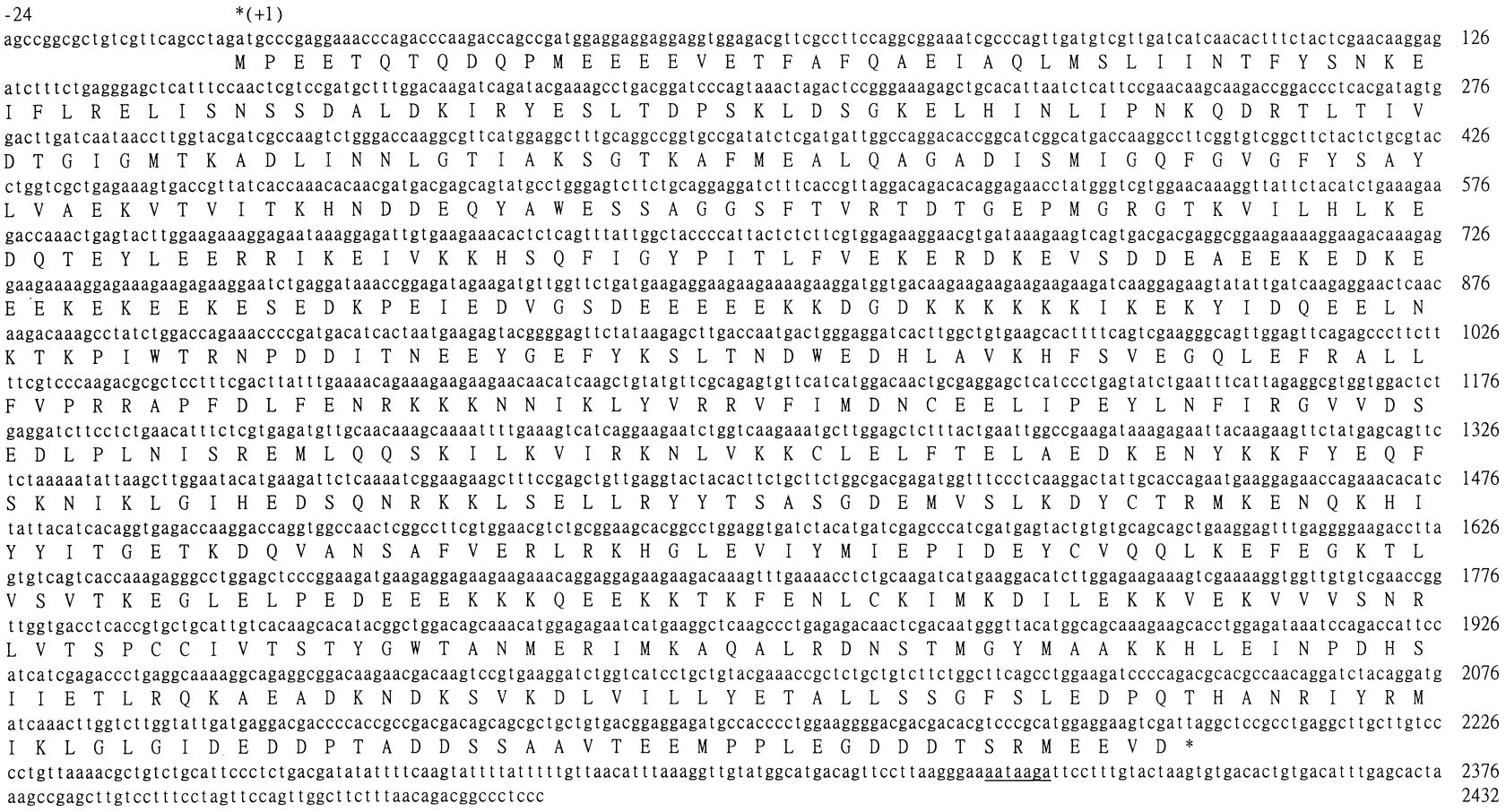

Fig. 2. Nucleotide and deduced amino acid sequence of the cDNA encoding porcine *hsp90*. The amino acid sequence is shown in the single-letter code below the nucleotide sequence. The start (+1) and stop codons are marked by asterisk. The putative polyadenylation signal (aataaga) residues is at nt 2329 (underlined). This sequence is available from GenBank under Accession No. U94395.

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                                                                                *
human   MPEETQTQDQ PMEEEEVETF AFQAEIAQLM SLIINTFYSN KEIFLRELIS NSSDALDKIR YETLTDPSKL DSGKELHINL   80
pig     MPEETQTQDQ PMEEEEVETF AFQAEIAQLM SLIINTFYSN KEIFLRELIS NSSDALDKIR YESLTDPSKL DSGKELHINL
mouse   MPEETQTQDQ PMEEEEVETF AFQAEIAQLM SLIINTFYSN KEIFLRELIS NSSDALDKIR YESLTDPSKL DSGKELHINL
chicken MPEAVQTQDQ PM.EEVETF AFQAEIAQLM SLIINTFYSN KEIFLRELIS NSSDALDKIR YESLTDPSKL DSGKDLKINL

human   IPNKQDRTLT IVDTGIGMTK ADLINNLGTI AKSGTKAFME ALQAGADISM IGQFGVGFYS AYLVAEKVTV ITKHNDDEQY  160
pig     IPNKQDRTLT IVDTGIGMTK ADLINNLGTI AKSGTKAFME ALQAGADISM IGQFGVGFYS AYLVAEKVTV ITKHNDDEQY
mouse   IPSKQDRTLT IVDTGIGMTK ADLINNLGTI AKSGTKAFME ALQAGADISM IGQFGVGFYS AYLVAEKVTV ITKHNDDEQY
chicken IPNKHDRTLT IVDTGIGMTK ADLVNNLGTI AKSGTKAFME ALQAGADISM IGQFGVGSYS AYLVAEKVTV ITKHNDDEQY

human   AWESSAGGSF TVRTDTGEPM GRGTKVILHL KEDQTEYLEE RRIKEIVKKH SQFIGYPITL FVEKERDKEV SDDEAEEKED  240
pig     AWESSAGGSF TVRTDTGEPM GRGTKVILHL KEDQTEYLEE RRIKEIVKKH SQFIGYPITL FVEKERDKEV SDDEAEEKED
mouse   AWESSAGGSF TVRTDTGEPM GRGTKVILHL KEDQTEYLEE RRIKEIVKKH SQFIGYPITL FVEKERDKEV SDDEAEEKEE
chicken AWESSAGGSF TVRLDNGEPL GRGTKVILHL KEDQTEYLEE RRIKEIVKKH SQFIGYPIRL FVEKERDKEV SDDEAEE...
                                  *
human   KEEEKEKEEK ESEDKPEIED VGSD.EEEEK KDGDKKKKKK IKEKYIDQEE LNKTKPIWTR NPDDITNEEY GEFYKSLTND  320
pig     KEEEKEKEEK ESEDKPEIED VGSDEEEEEK KDGDKKKKKK IKEKYIDQEE LNKTKPIWTR NPDDITNEEY GEFYKSLTND
mouse   KEEEKEKEEK ESDDKPEIED VGSDEEEEEK KDGDKKKKKK IKEKYIDQEE LNKTKPIWTR NPDDITNEEY GEFYKSLTND
chicken KEEEKEEKEE KTEDKPEIED VGSD.EEEEK KDGDKKKKKK IKEKYIDEEE LNKTKPIWTR NPDDITNEEY GEFYKSLTND

human   WEDHLAVKHF SVEGQLEFRA LLFVPRRAPF DLFENRKKKN NIKLYVRRVF IMDNCEELIP EYLNFIRGVV DSEDLPLNIS  400
pig     WEDHLAVKHF SVEGQLEFRA LLFVPRRAPF DLFENRKKKN NIKLYVRRVF IMDNCEELIP EYLNFIRGVV DSEDLPLNIS
mouse   WEEHLAVKHF SVEGQLEFRA LLFVPRRAPF DLFENRKKKN NIKLYVRRVF IMDNCEELIP EYLNFIRGVV DSEDLPLNIS
chicken WEDHLAVKHF SVEGQLEFRA LLFVPRRAPF DLFENRKKKN NIKLYVRRVF IMDNCEELIP EYLNFMRGVV DSEDLPLNIS

human   REMLQQSKIL KVIRKNLVKK CLELFTELAE DKENYKKFYE QFSKNIKLGI HEDSQNRKKL SELLRYYTSA SGDEMVSLKD  480
pig     REMLQQSKIL KVIRKNLVKK CLELFTELAE DKENYKKFYE QFSKNIKLGI HEDSQNRKKL SELLRYYTSA SGDEMVSLKD
mouse   REMLQQSKIL KVIRKNLVKK CLELFTELAE DKENYKKFYE QFSKNIKLGI HEDSQNRKKL SELLRYYTSA SGDEMVSLKD
chicken REMLQQSKIL KVIRKNLVKK CLELFTELAE DKENYKKFYE QFSKNIKLGI HEDSQNRKKL SELLRYYTSA SGDEMVSLKD

human   YCTRMKENQK HIYYITGETK DQVANSAFVE RLRKHGLEVI YMIEPIDEYC VQQLKEFEGK TLVSVTKEGL ELPEDEEEKK  560
pig     YCTRMKENQK HIYYITGETK DQVANSAFVE RLRKHGLEVI YMIEPIDEYC VQQLKEFEGK TLVSVTKEGL ELPEDEEEKK
mouse   YCTRMKENQK HIYFITGETK DQVANSAFVE RLRKHGLEVI YMIEPIDEYC VQQLKEFEGK TLVSVTKEGL ELPEDEEEKK
chicken YCTRMKENQK HVYYITGETK DQVANSAFVE RLRKHGLEVI YMIEPIDEYC VQQLKEFEGK TLVSVTKEGL ELPEDEEEKK

human   KQEEKKTKFE NLCKIMKDIL EKKVEKVVVS NRLVTSPCCI VTSTYGWTAN MERIMKAQAL RDNSTMGYMA AKKHLEINPD  640
pig     KQEEKKTKFE NLCKIMKDIL EKKVEKVVVS NRLVTSPCCI VTSTYGWTAN MERIMKAQAL RDNSTMGYMA AKKHLEINPD
mouse   KQEEKKTKFE NLCKIMKDIL EKKVEKVVVS NRLVTSPCCI VTSTYGWTAN MERIMKAQAL RDNSTMGYM. AKKHLEINPD
chicken KQEEKKAKFE NLCKIMKDIL EKKVEKVVVS NRLVTSPCCI VTSTYGWTAN MERIMKAQAL RDNSTMGYMA AKKHLEINPD
                                                                                *
human   HSIIETLRQK AEADKNDKSV KDLVILLYET ALLSSGFSLE DPQTHANRIY RMIKLGLGID EDDPTADDTS AAVTEEMPPL  720
pig     HSIIETLRQK AEADKNDKSV KDLVILLYET ALLSSGFSLE DPQTHANRIY RMIKLGLGID EDDPTADDSS AAVTEEMPPL
mouse   HSIIETLRQK AEADKNDKSV KDLVILLYET ALLSSGFSLE DPQTHANRIY RMIKLGLGID EDDPTVDDTS AAVTEEMPPL
chicken HSIIETLRQK AEADKNDKSV KDLVILLYET ALLSSGFSLE DPQTHANRIY RMIKLGLGID EDDTAAEEAS PAVTEEMPPL

human   EGDDDTSRME EVD*  734
pig     EGDDDTSRME EVD*
mouse   EGDDDTSRME EVD*
chicken EGDDDTSRME EVD*
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Fig. 3. Alignment of the deduced amino acid sequences of *hsp90* from human, pig, mouse, and chicken. Residue differences between human and pig are indicated by asterisks. Amino acid sequence of the human, mouse, and chicken are from Yamazaki et al. (1989), Moore et al. (1989), and Binart et al. (1989), respectively.

only three amino acid differences. These consisted of a glutamic acid insertion at position 265, and two threonine substitutions by serine at positions 63 and 709 when comparing pig to human sequences. By comparison, there were nine amino acid differences between human *hsp90* and mouse *hsp86*; the identity was 99.04%. There were eight amino acid differences between porcine *hsp90* and mouse *hsp86* with an identity of 99.04%.

3.2. Expression of porcine hsp90 in cultured smooth muscle cells and various porcine tissues

To test the heat inducibility of *hsp90* in cells, the level of *hsp90* mRNA in the control and heat-treated cells were compared by RT-PCR. Porcine aortic SMC were heat-shocked at 45°C for 60 min, then allowed to recover in normal culture condition varying from 0 to 8 h (Fig. 4). The mRNA was synthesized at a very low rate in the controls similar to that in the cells with no recovery time after hyperthermia. In contrast, the *hsp90* mRNA was expressed de novo after the cells were allowed to recover for 2 h, and this induction persisted for at least 8 h.

A Northern blot analysis was performed to confirm the above observation. Samples of cultured cells and various porcine tissues were collected from experiments in which the cells or animals were administrated with or without heat treatments. As expected, the basal levels

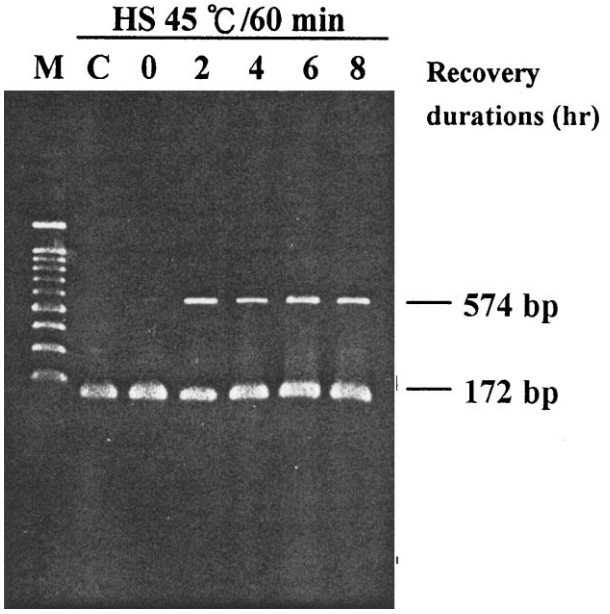

Fig. 4. Agarose gel electrophoresis of products from RT-PCR of heat shocked cells using *hsp90* cDNA derived primers. The cells were heat-shocked at 45°C for 60 min and then recovered from 0 to 8 h. The numbers on the right indicate products of *hsp90* (574 bp) and of *β*-actin (172 bp). C, control sample; M, size markers of 100-bp ladder (2000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp). Samples were electrophoresized on a 2% agarose gel.

of *hsp90* mRNA in control cells or tissues were barely detectable, but the amount of mRNA was greatly increased in cells or tissues after heat treatment (Fig. 5). In contrast, the expression of *hsp90* in brain decreased markedly with hyperthermia. Furthermore, three transcripts of 2.2, 3.0, and 4.1 kb in size were detected in both cultured cells and tissues.

3.3. HSP90 protein expression in cells and various tissues

To examine levels of the basal expression of HSP90 protein in various porcine tissues, polyclonal antibody against porcine HSP90 was used in a Western blot analysis. A higher amount of HSP90 was evident in pituitary gland, brain, adrenal gland, and testis (Fig. 6). Fig. 6 also showed that the HSP90 level was enhanced in SMC after heat treatments. However, HSP90 levels were not increased in the porcine tissues obtained 24 h after hyperthermia (data not shown).

4. Discussion

The HSP90 family is a group of abundantly expressed and highly conserved molecular chaperones whose exact function is presently undefined. They recognize and regulate the activity of several intracellular substrates, and also operate in the absence of stress (Jakob and Buchner, 1994). Mammalian HSP90 has two isoforms, HSP90*α* and HSP90*β*, which are encoded by separate genes (Moore et al., 1989). Both of the isoforms are expressed at basal levels under unstressful conditions; various stresses increase the expression of the two isoforms to different degrees. Hydrodynamic studies and molecular mass determination by SDS–polyacrylamide gel electrophoresis of HSP90 revealed that HSP90 exists mainly as monomers or dimers in the native state (Koyasu et al., 1986). Most of the dimeric HSP90 molecules are homodimers, either *α*/*α* or *β*/*β*, and the monomeric HSP90 molecules are mostly the *β* isoform (Minami et al., 1991). It is not clear whether the two isoforms are functional differently.

In the present study, we screened a porcine brain cDNA library with a human *hsp90α* probe. The amino acid sequence identity between the human *hsp90α* and the deduced porcine *hsp90* was 99.7%. However, the amino acid sequence of human HSP90α is 85% homologous to HSP90*β* (Rebbe et al., 1987; Hickey et al., 1989). Human *hsp90α* contains the glutamine-rich sequence (TQTQDQ), whereas human *hsp90β* does not. Furthermore, the sequence PEETQTQDQPME at the amino terminus of the human *hsp90α* is phosphorylated by the dsDNA-activated protein kinase (Lees-Miller and Anderson, 1989). Lacking both amino-terminal threonines, *hsp90β* cannot be phosphorylated. The deduced amino acid sequence of pig *hsp90* from our study contains the same sequence PEETQTQDQPME at the amino terminus. Thus, the clones obtained were most likely to be porcine *hsp90α*. The coding sequences of the *hsp90α* gene have been highly conserved during vertebrate evolution. According to the molecular evolutionary tree of HSP90-related proteins (Moore et al., 1989), the porcine HSP90 and human HSP90 were given exactly equal branch lengths.

We further compared the amino acid sequence of pig and human HSP90. A deletion was found in human HSP90 at Glu^{265}, and two substitutions of Thr to Ser residue at 63, and 709 were present in pig HSP90 (Fig. 3). The Glu^{265} deletion is also presented in the chicken HSP90 but not in the mouse. In view of similar hydrophilic characteristics of both threonine and serine, the substitution for each other may not alter the main structure and formation of HSP90. Two major immunogenic domains of human HSP90α have been identified, a highly charged region (residue 227–310) and a region near to the C terminus (residue 702–716) (Nemoto et al., 1997). Since the Ser/Thr substitutions do not change the net charge, the three-dimensional structure of the porcine HSP90 may be virtually the same as that of the human HSP90.

In this study, we also found that three transcripts of porcine *hsp90*, 2.2, 3.0, and 4.1 kb in size, were present in cells and tissues. The probe for the Northern hybridization was designed according to our porcine *hsp90* cDNA sequence, which is most likely to be *hsp90α*

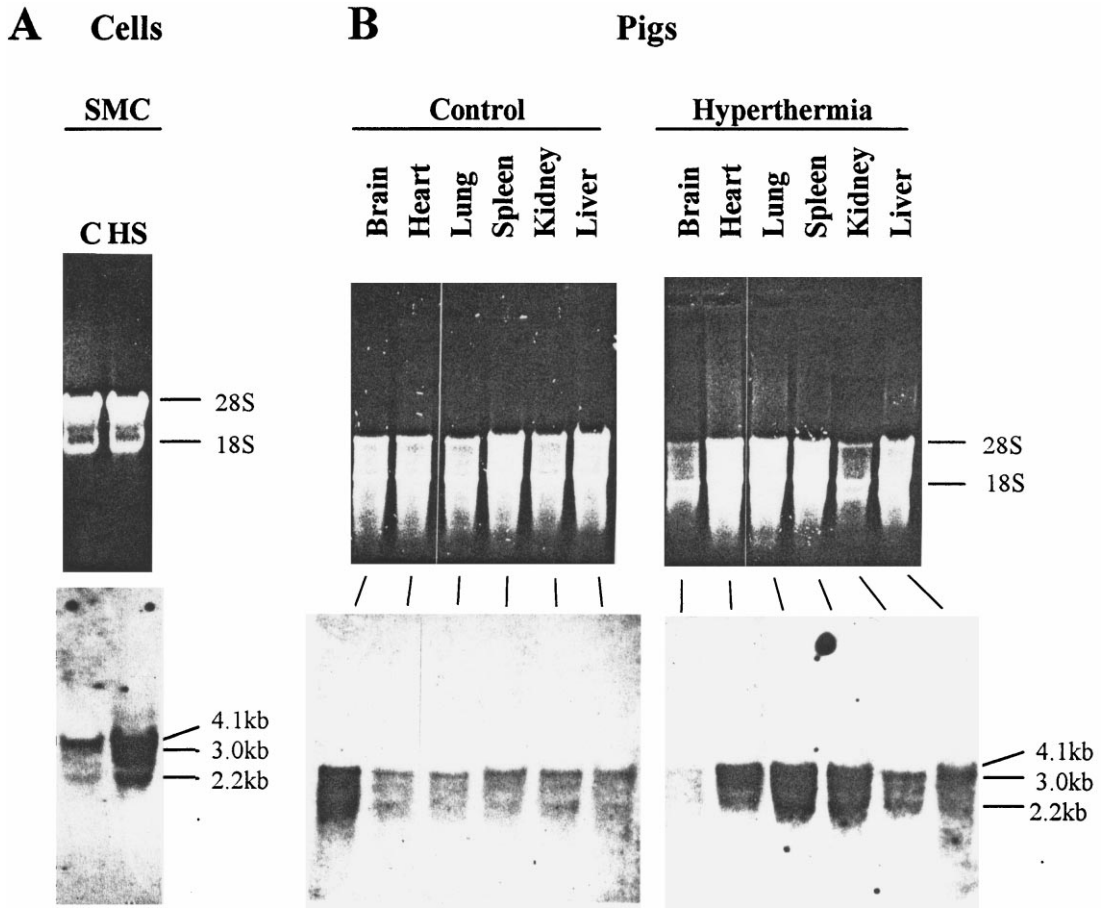

Fig. 5. Northern blot analysis of pig *hsp90* expression. Twenty micrograms of total RNA extracted from cells (**A**) and tissues (**B**) were loaded on each lane and resolved on a 1% formaldehyde agarose gel. RNA was visualized by ethidium bromide staining (upper figures). Both 28S and 18S are indicated. After transfer on to the nylon filters, the RNA was hybridized with biotinylated riboprobe in hybridization buffer at 65°C overnight followed by detection procedure (lower figures). Three transcripts were indicated: 2.2, 3.0, and 4.1 kb. C, control sample; HS, SMC heat-shocked at 45°C for 60 min and allowed to recover for 2 h; hyperthermia, the pig was heated to 43°C for 30 min and allowed to recover for 24 h. Brain, heart, lung, spleen, kidney, and liver are indicated.

(Fig. 5). In mouse testes, there are 3.2-kb *hsp90α* and 2.9-kb *hsp90β* transcripts (Gruppi et al., 1991); a 2.5-kb transcript of human *hsp90* (Rebbe et al., 1987) and a 3.4–3.5-kb transcript of mosquitoes *hsp90* (Su and Wellems, 1994) have also been reported. This pattern may be due to transcripts from other members of the *hsp90* family or to the alternative splicing of a single gene. Therefore, we cannot exclude the possibility that some signals detected in our Northern blots represented *hsp90β* transcripts.

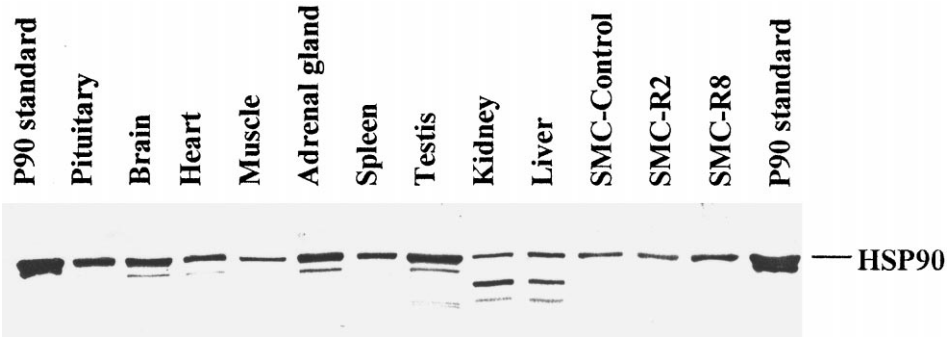

Fig. 6. Western blot analysis of porcine HSP90 from various tissues. Each lane containing 100 μg of total protein was performed on a 9% SDS–polyacrylamide gel. After electrophoresis, the proteins were transferred on to membrane and subsequently hybridized by using HSP90 polyclonal antibody as primary antibody. SMC, control, recovery at 2 and 8 h. The far left and right lanes are both purified human HSP90 to serve as references.

Tissue-specific differences in levels of constitutive HSP90 were observed. Our findings are in agreement with those found in rabbits (Quraishi and Brown, 1995). The HSP90 levels were lowest in muscle but highest in brain and testes (Fig. 6). Intermediate levels were detected in heart, kidney, liver, spleen, and lung. Following hyperthermia, induction of HSP90 was not significant in porcine tissues as detected by Western blotting. In contrast, HSP70 was increased to an abundant level in most tissues following hyperthermia (unpublished data). This result may have been obtained because the hyperthermia and recovery conditions were sufficient for *hsp90* transcription but not for translation. If the recovery time is prolonged, HSP90 would accumulate as a response to heat. The variation in constitutive high levels in brain, adrenal gland, pituitary, and testes may be due to varying sensitivities of tissues to steroid hormones, as suggested by Vamvakopoulos (1993). A further study of the expression and regulation of porcine *hsp90* in cells and whole animals is warranted.

In summary, we have obtained two cDNA clones encoding porcine *hsp90* sequence and demonstrated that hyperthermia increases the expression of this gene in cell culture and whole animals. The almost identical sequence of the HSP90 polypeptide between human and pig allows investigators to study its physiological functions in vivo. This subject also provides a new opportunity to study the pathogenesis of cardiovascular diseases such as the sudden-death pig model with HCM.

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