Characterization of a Novel Mammalian Co-chaperone, DnaJB7, an HSP40 Family Member

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Abstract : Molecular chaperones of HSP70 family members usually work together with HSP40/DNAJ family members in protein biogenesis and protein homeostasis. In mammals, approximately 50 members of the HSP40/DNAJ family are identified so far. Here, we isolated a cDNA clone of DnaJB7, one of the HSP40/DNAJ family members, and examined the properties of DnaJB7 protein *in vivo* and *in vitro*. Since the *DnaJB7* gene in the human genome has no obvious heat shock element (HSE), DnaJB7 could not be induced by heat shock in HeLa cells. While overexpressed DnaJB7 in HeLa cells was distributed to both the cytoplasm and nucleolus at normal temperature, upon heat shock it accumulated in the nucleolus, where it co-localized with Hsp70. This localization pattern was very similar to that of Hsp40 (DnaJB1). Direct interaction of Hsp70 with DnaJB7 in HeLa cells was demonstrated by immunoprecipitation. Also, DnaJB7 could stimulate the ATPase activity of Hsp70. Hsp70 had molecular chaperone activity in suppressing aggregation of heat denatured luciferase, and DnaJB7 could facilitate the chaperone activity of Hsp70. In murine tissues, DnaJB7 was highly expressed in the stomach and faintly in pancreas, uterus and ovary. These results suggest that DnaJB7 is a stomach-enriched co-chaperone of Hsp70.

Key Words : molecular chaperone, HSP40/DNAJ, co-chaperone, DnaJB7

Introduction

Heat shock protein 70 (HSP70) family members function as molecular chaperones and usually act together with HSP40/DNAJ family members in various aspects of the life of proteins, such as protein folding, assembly, secretion, and degradation¹⁻⁴). HSP40 family proteins have three distinct domains : (1) a highly conserved J domain of approximately 70 amino acids containing the family signature HPD (His-Pro-Asp) motif that interacts with the ATP binding domain of HSP70 to stimulate its ATPase

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activity; (2) a Gly and Phe (G/F) -rich region possibly acting as a flexible linker; and (3) a zinc-finger-like domain containing a Cys-rich region with several [CXXCXGXG] motifs⁵). The HSP40 family members can be classified into three different subtypes. Type I HSP40s (such as bacterial DnaJ, yeast Ydj1, and mammalian DnaJA1) have all three domains (J, G/F, and C); Type II members (such as yeast Sis1 and mammalian DnaJB1) have J and G/F domains but not the C domain; Type III members contain the J domain alone with other specialized domains that enable them to interact with specific substrates^{6–8}).

To date, approximately 50 members of the HSP40 family have been identified in the mammalian genome, 5 members in Type I, 14 in Type II, and 32 in Type III^{9,10}). Although more than ten members of this family have been characterized, most of the other members have not yet been studied. We here chose DnaJB7 for investigation, because it is a member of the Type II group, as is Hsp40 (DnaJB1) that we originally isolated, and has not yet been characterized. We characterized DnaJB7 protein *in vivo* and *in vitro*.

Materials and methods

DNA construct, protein purification and antibody production

Nucleotide and amino acid sequences of DnaJB7 were found in the NCBI database (Ac. No. NM_145174.1 and NP_66015, respectively). We isolated a cDNA clone of DnaJB7 from total RNA extracted from human HeLa cells by the RT-PCR method, with 5'-TATTTGCAGTCACTACCTC TATCACC-3' as a forward primer, and 5'-CTCATCCTTTTCTGACATACCCATTC-3' as a reverse primer. The isolated cDNA comprised 1203 base pairs and was confirmed as DnaJB7 by nucleotide sequencing (BigDye Terminator V1.1 Cycle sequencing kit, Applied Biosystems, Foster City, CA, USA). To create His-tagged, FLAG-tagged, and GST-fusion versions of DnaJB7, the isolated cDNA was subcloned into pET30a+ (Novagen, Darmstadt, Germany), pFLAG-CMV-6c (Sigma, St. Louis, MO, USA), and pGEX-3X (GE Healthcare, Chalfont St. Giles, UK) vectors, respectively.

The His-tagged DnaJB7 recombinant protein was expressed in *E. coli*, BL21 (DE3) strain, according to the manufacturer's protocol (QIAexpress System, Qiagen, Valencia, CA, USA). Cells were harvested after a 2-hr induction at 37°C, and disrupted by sonication in lysis buffer (8 M urea, 100 mM NaH₂PO₄, 10 mM Tris-HCl, pH 8.0). The recombinant protein was purified by using Ni²⁺-NTA-agarose beads (Qiagen). The purified His-DnaJB7 protein was used as an antigen to raise anti-DnaJB7 anti-serum in a rabbit by standard methods (immunization with 1 mg of protein four times every two weeks). Since the obtained anti-serum reacted with various non-specific proteins in an *E. coli* extract, the IgG fraction in the anti-serum was first affinity-purified by MAbTrapTM (GE Healthcare), and then by antigen-column. In brief, the purified His-tag DnaJB7 protein was subjected to SDS-PAGE (polyacrylamide gel electrophoresis) on a preparative slab gel and blotted to a nitrocellulose membrane. The membrane was stained with Ponceau S (Wako Pure Chemicals, Osaka, Japan) to detect DnaJB7 protein, and the protein portion of the membrane was cut into about 1 mm×1 mm pieces. The membrane pieces were then washed with TBS, mixed with the affinity-purified IgG fraction, and then rotated overnight at 4°C. The membrane pieces were washed with PBS, and the bound antibody was eluted with 0.5 M glycine. The pH of the eluted solution was adjusted at 7.0 with 1 M Tris-HCl (pH 8.0)

and then dialyzed against TBS. The purified antibody solution was stored at -30°C.

The GST-DnaJB7 and GST-Hsp40 recombinant proteins were expressed in *E. coli*, BL21 (DE3) strain, and purified with GSTrap FF according to the manufacturer's protocol (GE Healthcare).

Cell culture, animals, and reagents

HeLa cells derived from human cervical cancer were grown in Dulbecco's modified Eagle's minimal essential medium (D-MEM, GIBCO, Grand Island, NE, USA) supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% penicillin-streptomycin (GIBCO), and incubated in a CO₂ incubator with 5% CO₂ and 95% air at 37°C. When indicated, cells were heated by immersing the culture dishes sealed with Parafilm into a temperature-controlled water bath. Male ICR mice (5 weeks old) were purchased from SLC, Ltd. (Shizuoka, Japan), and kept under conventional conditions (room temperature 22°C, 12 hr dark/light cycle) for 1 week before harvesting of various tissues. Most of the restriction enzymes used were purchased from Takara Co. Ltd. (Ohtsu, Japan). Other reagents were of the highest purity available from Wako Pure Chemicals and Nacalai Tesque (Kyoto, Japan).

Immunological methods and gel electrophoresis

For Western blotting, cells, protein samples or mouse tissues were dissolved in sodium dodecyl sulfate (SDS) sample buffer (2.3% SDS, 62.5 mM Tris-HCl [pH 6.8], 5% β-mercaptoethanol, 10% glycerol) and boiled for 5 min. The protein concentration of each sample was determined with a Pierce protein assay kit (Pierce, Rockford, IL, USA). Samples of protein (5 to 20 μg) were separated by standard SDS polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes (Schleicher & Schnell, Dassel, Germany). The blotted membranes were probed with the first antibody (1/100 to 1/2,000 dilutions). The first antibodies used in this study were anti-DnaJB7 (this study), anti-Hsp70 (mouse monoclonal, SPA-810, StressGen, Victoria, Canada), anti-Hsp40¹¹⁾, anti-His-tag (2366S, CST JAPAN, Tokyo, Japan), and anti-luciferase (G745A, Promega, Fitchburg, WI, USA) antibodies. The membranes were then probed with the horseradish peroxidase-conjugated corresponding immunoglobulin G (IgG; Zymed, San Francisco, CA, USA). They were then treated with Amersham ECL Western Blotting Detection Reagents (GE Healthcare), and signals were detected by VersaDoc™ Model 5000 (BIO-RAD, Hercules, CA, USA). The relative signal intensity was quantified by densitometry with NIH Image software (Scion Image) on a personal computer.

For the analysis of the intracellular localization of DnaJB7, HeLa cells were grown on glass coverslips (9 mm × 9 mm) in 35 mm dishes. Cells were transfected with the pFLAG-6c-DnaJB7 plasmid using Lipofectoamine (Invitrogen, Carlsbad, CA, USA) and cultured in FBS-free D-MEM overnight. The culture medium was then replaced with fresh medium (D-MEM with 10% FBS) and further cultured for 24 hr. The non-treated control or heat-shocked cells on a coverslip were washed with PBS and fixed in 100% methanol at -30°C for 10 min. The cells were then incubated with 10% normal goat serum (Sigma) to inhibit nonspecific binding followed by a mixture of anti-Hsp70 (rabbit polyclonal antibody, KO4)¹²⁾ and anti-FLAG (mouse monoclonal, F3165, Sigma) antibodies, or a mixture of anti-Hsp70 (mouse monoclonal, SPA-810, StressGen) and anti-DnaJB7 (rabbit polyclonal) antibodies. The second antibody was a mixture of rhodamine-conjugated anti-rabbit IgG and FITC-conjugated goat anti-mouse

IgG, or a mixture of FITC-conjugated goat anti-mouse IgG and rhodamine-conjugated anti-rabbit IgG. Cells were observed through a Fluorophoto microscope (Nikon, Tokyo, Japan).

Interaction between Hsp70 and DnaJB7 or Hsp40 was investigated by immunoprecipitation. We used HeLa cells transfected with FLAG-tagged DnaJB7. After the cells were harvested and washed with PBS, they were resuspended in 2 mM DSP [3,3'-dithiobis- (succinimidyl propionate), cleavable cross linker, see ref. 12] in PBS and incubated for 30 min at room temperature with gentle shaking. The reaction of DSP was stopped by the addition of 2 mM glycine. The cells were again washed with PBS and disrupted by sonication in lysis buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulfonylfluoride, 2% protease inhibitor cocktail [Sigma]). The cell lysates were clarified by centrifugation (15,000 rpm for 30 min at 4°C). The supernatants were incubated overnight with anti-Hsp70 (mouse monoclonal, SPA-810 StressGen) or anti-DnaJB7 antibody at 4°C with rotation, and then Protein-G Sepharose beads (GE Healthcare) were added and rotation was continued overnight at 4°C. The beads were washed with lysis buffer, suspended in SDS-sample buffer, and then boiled for 5 min. Samples were analyzed by Western blotting with anti-Hsp70 (SPA-810, StressGen) or anti-DnaJB7 antibodies.

ATPase assay

Hsp70 ATPase activity was measured by monitoring the concentration of phosphate released in the reaction by using the Malachite Green Phosphate Assay Kit (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacture's instructions. Purified recombinant Hsp70 was purchased from StressMarQ (SPR-108B, the cDNA was originally cloned from a human cDNA library and expressed in *E. coli*). GST, GST-DnaJB7, and GST-Hsp40 were purified as described above and dialyzed overnight against ATPase assay buffer (50 mM HEPES, pH 6.8, 50 mM NaCl, 2 mM MgCl₂). Assays were performed in a final volume of 100 μ l consisting of ATPase buffer with 20 mM KCl and 20 μ M ATP. Reactions contained 0.75 or 1.5 μ M Hsp70 in the presence or absence of GST or GST-DnaJB7 at indicated concentrations. After incubation at 25°C for 75 min, the concentration of phosphate in the reaction mixture was measured.

Luciferase aggregation assay

Luciferase aggregation assay was performed essentially according to the same protocol described in Fan et al¹³. In brief, luciferase (50 nM, Promega) was mixed with Hsp70 (1.5 μ M) and GST-DnaJB7 or GST-Hsp40 in a final volume of 20 μ l containing ATPase buffer with 20 mM KCl, 2 mM ATP, and 2 mM dithiothreitol. The reaction mixtures were heated at 42°C for 15 min, and then centrifuged at 15,000 rpm for 20 min at 4°C. Total (T) luciferase and luciferase that was present in the supernatant (S) and pellet (P) from equal portions of reaction mixture were then determined by Western blotting with anti-luciferase antibody (Promega).

Results

Isolation of DnaJB7 cDNA clone and production of anti-DnaJB7 antibody

We first isolated the DnaJB7 cDNA clone from total RNA extracted from human HeLa cells by RT-PCR methods. Nucleotide sequencing of DnaJB7 cDNA revealed that it encodes a protein of 308 amino acid residues with a molecular mass of 35,433. The deduced amino acid sequence showed that DnaJB7 protein is a Type II member of the HSP40 family with J and G/F domains (Fig. 1, Ac. No. NP_660157 in the NCBI protein database). Homology search among type II homologs revealed that DnaJB7 is the most homologous to DnaJB6 (Mrj) with 55% overall identity and 67% similarity (Fig. 1, analyzed by Blast in the EMBL-EBI database).

DnaJB7	1	MVDYYEVLGLQRYASPEDIKKAYHKVALKWHDPKNPENKEEAERKFKFEVAEAYEVLSDNE	60
		MVDYYEVLG+QR+ASPEDIKKAY K+ALKWHPDKNPENKEEAERKFK+VAEAYEVLSD+	
DnaJB6	1	MVDYYEVLGVQRHASPEDIKKAYRKALKWHDPKNPENKEEAERKFKQVAEAYEVLSDAK	60
	61	KRDIYDKYGTGLN---GGGSHFDDECEYGFTHKPDVDFKEIFHERDPFSFHFEDSLE	117
		KRDIYDKYG EGLN GGGSHFD E+GFTF PDDVF+E F RDPFSF FFED E	
	61	KRDIYDKYKGEGLNGGGGGSHFDSPFEGFTFRNPDDVFRFFGGRDPFSDFFEDPFE	120
	118	DLLNRPGSSYGNNRNDAGYFFSTASEYPIF-EKFSSYDTGYTSQGSGLGHEGLTSFSSLA	176
		D G+R+R G FFS S +P F FSS+DTG+TS GSLGH GLTSFSS +F	
	121	DFFGNRRGPRGSRSGTGSFFSAFSGFPSPGSGFSSFDTGFTSFGSLGHGGLTSFSSTSF	180
	177	DNSGMDNYISVTTSDKIVNGRNINTKKIIESDQER-EAEDNGELTFFLVNSVANEEGFAK	235
		SGM N+ S++TS K+VNGR I TK+I+E+ QER E E++G+L +N VA+++ A	
	181	GGSGMGNFKSIISTSTMVNGRKITTKRIVENGQERVEVEEDGQLKSLTINGVADDALAE	240
	236	ECSWRTQSFNNYSP-----NSHSSKHVSQYTFVDNDEGGISWVTSNRDPPIFSAG	285
		E R Q+ P + +H + E DP +AG	
	241	ERMRRGQNALPAQPAGLRPPKPPRPASLLRHAPHCLSEEEGEQDRPRAPGPWDPLASAAG	300
	286	VKEGGKRKKKKRKE--VQKKSTKRN	308
		+KEGGKRKK+K++E +KKSTK N	
	301	LKEGGKRKKQKQREESKKKKSTKGN	325

Fig. 1. Deduced amino acid sequence of DnaJB7 and sequence comparison between DnaJB7 and DnaJB6 (Mrj) (indicated as one-letter code). The first line is the amino acid sequence of DnaJB7 and the third line is that of DnaJB6. The second line indicates identical or similar (+) amino acid residues.

The His-tagged DnaJB7 protein was then expressed in *E. coli* and purified with a Ni-NTA agarose column. The purified protein was used as the antigen for rabbit immunization. Anti-serum was obtained and checked for specificity of the antigen-antibody reaction by Western blotting. As shown in Fig. 2B, the anti-serum detected many non-specific proteins in the extract of *E. coli*. Therefore, we first purified the antibody by protein-G affinity column. The partially purified antibody reacted not only

with DnaJB7 but also with a non-specific band around 90-kDa (Fig. 2D). We then purified the antibody by using an antigen column as described in Materials and methods. The purified antibody specifically detected His-tag and GST-tag DnaJB7 with virtually no apparent non-specific noise signals (Fig. 2E). We used this purified antibody in the following experiments.

Fig. 2. Specificity of anti-DnaJB7 antibody. (A) Cell extracts of *E. coli* were subjected to SDS-PAGE and stained with Coomassie blue. Lane 1, cells containing His-tagged DnaJB7 plasmid (not induced); lane 2, His-tagged DnaJB7 was induced; lane 3, cells containing GST-fused DnaJB7 plasmid (not induced); lane 4, GST-fused DnaJB7 was induced. The arrowhead indicates His-tagged DnaJB7, and arrow GST-fused DnaJB7. Molecular mass markers are shown at the left side in kilodaltons. (B) Western blot probed with rabbit anti-DnaJB7 antiserum. Note that there are many non-specific signals. (C) Western blot probed with anti-His-tag antibody. Only His-tagged DnaJB7 was detected in lane 2. (D) Western blot probed with anti-DnaJB7 antibody purified by a protein-G affinity column. His-tagged DnaJB7 in lane 2 and GST-DnaJB7 in lane 4 were detected. In addition, a non-specific band with molecular mass of about 90-kDa was detected. (E) Western blot probed with anti-DnaJB7 antibody purified by using an antigen-column as described in Materials and methods. His-tagged DnaJB7 in lane 2 and GST-DnaJB7 in lane 4 were specifically detected.

DnaJB7 was not induced by heat shock stress

Since we could not find a distinct heat shock element (HSE), which is an essential *cis*-acting base sequence for induction by heat stress, within the promoter region of the human DnaJB7 gene (located at chromosome 22q13.2), we examined the inducibility of DnaJB7 by heat shock in HeLa cells by Western blotting. As shown in Fig. 3, DnaJB7 was not induced by heat shock. In contrast, classical Hsp70 (HSPA1A) and Hsp40 (DnaJB1) were clearly induced by heat stress. Since the expression level of DnaJB7 in HeLa cells was too low to detect it by Western blotting, the signal was very weak with high background (Fig. 3, panel of DnaJB7). Therefore, we used an overexpression system for DnaJB7 in the following studies.

Fig. 3. Inducibility of DnaJB7 protein by heat shock in HeLa cells. HeLa cells at subconfluency were heated at 42°C for 2 hr and then allowed to recover for 24 hr at 37°C. (A) Western blot probed with anti-DnaJB7, anti-Hsp40, and anti-Hsp70 (mouse monoclonal, SPA-810, StressGen) antibodies. Lane 1, non-treated control cells; lanes 2-8, cells were allowed to recover for 0, 1, 2, 4, 8, 12, and 24 hr after heating, respectively. The panel under the Western blot shows the corresponding SDS-PAGE gel stained with Coomassie blue around a 43-kDa protein as the protein loading control. (B) Relative amounts of DnaJB7, Hsp40, and Hsp70 were measured by densitometric analysis of each band of the Western blot in (A). Data are shown as the average of relative expression $\pm$ SD of three independent experiments.

Intracellular localization of DnaJB7

The intracellular distribution of DnaJB7 was examined in HeLa cells in which FLAG-tagged DnaJB7 was overexpressed. In non-heat-shocked control cells, DnaJB7 (stained with anti-FLAG antibody) was seen in both the cytoplasm and nuclei (including nucleoli) (Fig. 4A, panel a) and Hsp70 was diffusely located mainly in the cytoplasm (Fig. 4A, panel b), indicating co-localization of DnaJB7 and Hsp70 in the cytoplasm at 37°C (Fig. 4A, panel c). In contrast, when the cells were heated at 43°C for 30 min, both DnaJB7 and Hsp70 were translocated into the nuclei, especially in the nucleoli (Fig. 4A, panels e and f), where both proteins co-localized (Fig. 4A, panel g). The localization pattern of DnaJB7 is very similar to that of Hsp40 (DnaJB1)^{11,14}. Similar results were obtained when the cells were stained with anti-DnaJB7 antibody (Fig. 4B).

Fig. 4. Intracellular localization of DnaJB7 and Hsp70 in HeLa cells in which FLAG-tagged DnaJB7 was overexpressed. (A), cells growing at 37°C (a, b, c, and d), or heated at 43°C for 30 min (e, f, g, and h) were fixed and processed for immunofluorescent staining. Panels a and e, cells stained with anti-FLAG antibody; panels b and f, cells stained with anti-Hsp70 antibody (rabbit polyclonal KO4); panel c, merged image of panels a and b; panel g, merged image of panels e and f; panels d and h, phase contrast images of each corresponding panel. (B), cells growing at 37°C (a, b, c, and d), or heated at 43°C for 30 min (e, f, g, and h) were fixed and processed for immunofluorescent staining. Panels a and e, cells stained with anti-DnaJB7 antibody; panels b and f, cells stained with anti-Hsp70 antibody (SPA-810, StressGen); panel c, merged image of panels a and b; panel g, merged image of panels e and f; panels d and h, phase contrast images of each corresponding field.

Interaction of DnaJB7 with Hsp70

The direct interaction between DnaJB7 and Hsp70 was examined in DnaJB7-overexpressed HeLa cells by immunoprecipitation. As shown in Fig. 5A, anti-Hsp70 antibody could precipitate DnaJB7 (lane 5 in Fig. 5A), and anti-DnaJB7 antibody could precipitate Hsp70 (lane 7 in Fig. 5A). A similar interaction between Hsp70 and Hsp40 (DnaJB1) was observed in HSP-enriched HeLa cells (Fig. 5B).

We next investigated the effect of DnaJB7 on the ATPase activity of Hsp70. Hsp70 had a basal level of ATPase activity (column 4 in Fig. 6A), and this activity was significantly stimulated by DnaJB7 (column 6 in Fig. 6A). The stimulatory effect of DnaJB7 was similar to that of Hsp40 (DnaJB1) (column 6 versus 8 in Fig. 6A). Enhancement of Hsp70 ATPase activity by DnaJB7 was dependent on the concentration of DnaJB7 (Fig. 6B), and also dependent on the reaction time (Fig. 6C). Of course, either DnaJB7 or Hsp40 (DnaJB1) alone had no apparent ATPase activity (columns 3 and 7 in Fig. 6A).

Fig. 5. (A) Interaction of Hsp70 with DnaJB7 in HeLa cells in which FLAG-tagged DnaJB7 was overexpressed. Lanes 1, 4, 6, control HeLa cells; lanes 2, 3, 5, 7, DnaJB7 overexpressed cells; lanes 1 and 2, Western blot of whole cell lysates; lane 3, Protein-G Sepharose beads alone without antibody; lanes 4 and 5, cell lysates were immunoprecipitated with anti-Hsp70 antibody (SPA-810, StressGen); lanes 6 and 7, cell lysates were immunoprecipitated with anti-DnaJB7 antibody. (B) Interaction between Hsp70 with Hsp40 (DnaJB1) in HSP-enriched HeLa cells. The cells were heated at 42°C for 2 hr and then allowed to recover for 8 hr. Lane 1, Western blot of whole cell lysate of HSP-enriched HeLa cells; lane 2, Protein-G Sepharose beads alone without antibody; lane 3, cell lysates were immunoprecipitated with anti-Hsp70 antibody (SPA-810, StressGen); lane 4, cell lysates were immunoprecipitated with anti-Hsp40 antibody. Samples and immunoprecipitates were subjected to SDS-PAGE followed by Western blotting with anti-Hsp70 antibody (SPA-810, StressGen) or anti-Hsp40 antibody.

Fig. 6. Activation of Hsp70 ATPase by DnaJB7. (A) A recombinant Hsp70 (1.5 μM) was incubated with the indicated combinations of GST or GST-fused DnaJB7 or GST-fused Hsp40 at 25°C for 75 min, and the concentration of released phosphate was measured. (B) ATPase activity was measured at the indicated molar ratios (GST-DnaJB7/Hsp70). The concentration of Hsp70 was 1.5 μM (closed diamonds) or 0.75 μM (closed squares). (C) Time course of Hsp70 ATPase activity in the presence (closed diamonds) or absence (closed squares) of GST-DnaJB7. Data are presented as the average ± SD of three independent experiments.

Effect of DnaJB7 on the chaperone activity of Hsp70

We tested whether DnaJB7 could facilitate the chaperone activity of Hsp70 in suppressing heat-denatured protein aggregation. Upon heating of native firefly luciferase at 42°C for 15 min, more than 80% of it formed aggregates that could be pelleted from the reaction mixture by centrifugation (Fig. 7B, column 2). When Hsp70 was added to the reaction mixture during the heat treatment, luciferase aggregation was suppressed to less than 60% (Fig. 7B, column 3). The combination of Hsp70 and DnaJB7 led to enhanced suppression of luciferase aggregation (Fig. 7B, column 3 versus 5). A low molar ratio of DnaJB7 had no effect on the Hsp70 chaperone activity (Fig. 7B, column 3 versus 4). Of course, DnaJB7 alone had no chaperone activity (Fig. 7B, columns 6 and 7). In control experiments, we used Hsp40 (DnaJB1) and obtained almost the same results as with DnaJB7 (Fig. 7B, columns 8 to 11).

Fig. 7. Cooperation of Hsp70 with DnaJB7 (GST-tagged) in the suppression of heat-denatured luciferase. A, suppression of luciferase aggregation by Hsp70 and/or DnaJB7, and by Hsp70 and/or Hsp40 (DnaJB1). Details of the experimental procedures are described in the Materials and methods section. Typical Western blot data are shown. B, quantitation of luciferase present in the soluble fraction and pellets of the reaction mixture in panel A. Three independent experiments were performed. Differences between groups were compared by Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$.

Tissue distribution of DnaJB7 in mouse

We examined the distribution of DnaJB7 protein as well as classical Hsp70 (probed with rabbit polyclonal KO4 antibody) and Hsp40 (DnaJB1) in mouse tissues (Fig. 8). Hsp70 was expressed in all tissues examined, and Hsp40 was detected in the cerebrum, lung, spleen, uterus, ovary, and testis and faintly detected in the cerebellum, spinal cord, kidney, pancreas, stomach, and colon. Interestingly, DnaJB7 was highly expressed in the stomach and slightly in the pancreas, uterus, and ovary. This is the first report of a stomach-enriched HSP40/DNAJ family member.

Fig. 8. Tissue distribution of DnaJB7, Hsp40, and Hsp70 in mouse. Tissue extracts were subjected to Western blot analysis with anti-DnaJB7, anti-Hsp40, and anti-Hsp70 (rabbit polyclonal KO4) antibodies.

Discussion

Here, we have described the characterization of a novel mammalian HSP40 homolog, DnaJB7, a co-chaperone of Hsp70. DnaJB7 protein is a member of a type II HSP40 homolog, and its intracellular localization was very similar to that of Hsp40 (DnaJB1), which is induced by heat shock. Conversely, DnaJB7 was not induced by heat shock in HeLa cells probably because of the lack of an obvious heat shock element consensus sequence in the promoter region of the gene. DnaJB7 could stimulate Hsp70 ATPase activity and also enhance its chaperone activity of suppressing the aggregate formation of thermally denatured luciferase. Interestingly, DnaJB7 was highly expressed in mouse stomach. From these results, it is suggested that DnaJB7 is a stomach-enriched co-chaperone of Hsp70.

To date nearly 50 members of the HSP40 family have been identified in the mammalian genome^{9,10}. Among them, more than ten members have been studied. For example, DnaJA1 (Hsdj, Hdj2) has been shown to have essential roles in spermatogenesis and androgen receptor signaling¹⁵. DnaJA3 (Tid1) could reduce the malignant activity of an oncogene, ErbB-2, in carcinoma cells¹⁶. DnaJA4 is a co-chaperone enriched in mouse heart¹⁷. DnaJB1 (Hsp40, Hdj1) is the first identified mammalian DnaJ homolog that is induced by various environmental stresses^{18,11}. It has been reported to work together with Hsp70 and have chaperone activity *in vitro* and *in vivo*^{19–22}. Recently, it was demonstrated that DnaJB1 is predominantly expressed in the cytoplasm of spermatocytes, round spermatids, and elongating spermatids²³. DnaJB2 (Hsj1), which contains a ubiquitin-interacting motif, was able to reduce polyglutamine-mediated aggregate formation through target protein degradation by the ubiquitin-proteasome system²⁴. DnaJB6 (Mrj) has been reported to regulate the organization of keratin 8/18 intermediate filaments²⁵. Furthermore, DnaJB6 is enriched in the brain and could inhibit Huntingtin aggregation and toxicity²⁶, and has also been shown to be highly up-regulated in parkinsonian astrocytes²⁷. There are several HSP40/DNAJ homologs localized in the endoplasmic reticulum (named ERdj1 to ERdj5). Among them, DnaJB11 (ERdj3) has been reported to interact with BiP (ER-resident Hsp70) involving in protein folding in the endoplasmic reticulum²⁸. ERdj5 (DnaJC10) is another member of the ER-resident HSP40/DNAJ homologs, but it belongs to Type III and could function as disulfide reductase and accelerate ER-associated degradation of misfolded proteins²⁹. Cysteine string protein (Csp α , also called DnaJC5) is one of the Type III homologs, and is known to be anchored to synaptic vesicles as well as to exocrine and endocrine secretory granules³⁰. DnaJC13 (RME-8, receptor-mediated endocytosis-8) has been shown to be involved in endosomal trafficking^{31,32}. Sacsin (DnaJC29) is a huge protein with 4579 amino acid residues (approximately 520 kDa) and a causative gene product of spastic ataxia of Charlevoix-Saguenay, which is one on the neurodegenerative disorders. DnaJC29 has a J domain at the C-terminus and ubiquitin-like domain at the N-terminus. DnaJC29 is shown to be highly expressed in large neurons, particularly within brain motor systems, including cerebellar Purkinje cells³³.

While the deduced amino acid sequence of DnaJB7 (308 aa) is most homologous to that of DnaJB6 (Mrj) (325 aa) with 55% identity and 67% similarity (Fig. 1), the sequences of the J domain (N-terminal 74 aa) and C-terminal region (20 aa) of both are highly homologous, 88% and 70% identity (96% and 95% similarity), respectively (Fig. 1). These results suggest that DnaJB7 might work together with Hsp70 as a chaperone complex mainly in the stomach with involvement in intermediate filament

organization. Also, induction of HSPs, especially Hsp70, in mouse stomach by hyperthermia or some chemical compounds, such as geranylgeranylacetone (GGA) and paeoniflorin has been reported to have a protective effect on the gastric mucosal damage triggered by several irritants or ischemia/reperfusion oxidative stress^{34,35}. It has recently been shown that the protective effect of GGA on irritant-induced lesions was not observed in the heat shock factor 1 (HSF1) -null mutant mouse³⁶. Taken together, DnaJB7 might act as co-chaperone of Hsp70 in mouse stomach in protecting it from deleterious stresses which induce protein aggregation or misfolding.

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