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—Original—

Non-Hsp genes are essential for HSF1-mediated maintenance of whole body homeostasis

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Abstract: Mammalian tissues are always exposed to diverse threats from pathological conditions and aging. Therefore, the molecular systems that protect the cells from these threats are indispensable for cell survival. A variety of diseases, including neurodegenerative diseases, cause intracellular damage and disturb homeostasis. Heat shock transcription factor 1 (HSF1) positively regulates heat shock protein (Hsp) and maintains the precise folding of proteins. Moreover, HSF1 induces the non-Hsp genes expression, and degrades damaged/misfolded protein. Recently, my colleagues and I revealed non-Hsp genes have more protective roles than Hsps at the cellular level. However, whether these protective systems are similarly important to cellular defense in each tissue is still elusive. In this study, I compared polyglutamine (polyQ) protein aggregations/inclusion development in each tissue of WT- and HSF1KO-Huntington's disease (HD) mice, and examined the expression of the eight non-Hsp HSF1 target genes that have a strong suppressive effect on polyQ protein aggregation. Of these genes, Nfatc2, Pdzk3, Cryab, Csrp2, and Prame were detected in most tissues, but the other genes were not. Surprisingly, the obvious effect of HSF1 deficiency on the expression of these five genes was detected in only heart, spleen, and stomach. In addition, polyQ protein aggregations/inclusion was not detected in any tissues of WT-HD and HSF1KO-HD mice, but higher level of pre-aggregative polyQ protein was detected in HSF1KO-HD tissues. These results indicate non-Hsp genes are indispensable for the maintenance of intracellular homeostasis in mammalian tissues, resulting in whole body homeostasis.

Key words: non-Hsp, HSF1, homeostasis, aggregations/inclusion, polyQ

Introduction

All living things including mammalians are always exposed to various dangers disturbing intracellular and whole body homeostasis. Therefore, they possesses diverse mechanisms for survival against these dangers. In particular, neurodegenerative diseases-Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis–generate toxic intracellular insoluble aggregates and solid inclusions in neurons and glia cells. Consequently, similar aggregates and inclusions appear in other organs and tissues in addition to the central nervous system and disturb intracellular homeostasis, leading cell death.

Polyglutamine (polyQ) disease is also a neurodegenerative disease, but it is distinct from others, as polyQ disease is a generic name. Actually, nine disorders, Huntington's disease (HD), spinocerebellar ataxia 1 (SCA1), SCA2, dentatorubral-pallidoluysian atrophy (DRPLA), spinal bulbar muscular atrophy (SBMA), Machado-Joseph disease (SCA3), SCA6, SCA7, and SCA17 belong to this disease. These nine disorders develop abnormal conformational proteins resulting in toxic aggregates and inclusions, because their genes encode pathogenic polyQ

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proteins, having abnormally expanded CAG repeats in their exons. For example, huntingtin (HTT) protein is a cause of HD, the normal *HTT* gene has only ~40 CAG repeats in the genome. However, pathogenic *HTT* has more than 150 CAG repeats and generates pathogenic polyQ-HTT proteins containing more than 50 glutamine residues, and these polyQ-HTT proteins form aggregates and inclusion bodies. This characteristic feature is common to all nine polyQ diseases. However, as in the case of other neurodegenerative diseases, no therapeutics to cure these diseases have been developed.

As these aggregates and inclusions include misfolded proteins, the well-known chaperone heat shock protein (Hsp) has been expected to be useful for the development of effective medicine, because Hsp is a good chaperone and chaperone proteins have a strong ability to recover precise folding in misfolded proteins. Hsps bind to the nascent polypeptides released from ribosomes and assist in their precise folding, but it has recently been found that most intracellular proteins interact with Hsps and maintain their folding. In fact, Hsp90 have many client proteins and most intracellular proteins interact with Hsps [16]. Therefore, in the development of medicines for neurodegenerative diseases, Hsps have been the main targets for the discovery of useful therapeutics.

On the other hand, the induction and expression of Hsps are regulated by the transcription factor Heat shock transcription factor 1 (HSF1). HSF1 was originally discovered as a transcription factor of Hsp70 when cells were exposed to high temperature [18]. HSF1 is one of four mammalian HSFs, but only HSF1 has the ability to induce various Hsps. Hsp70 induction by heat shock is completely abolished in HSF1-deficient mouse embryonic fibroblasts (MEFs) [11]. Benjamin and his colleagues established HSF1-deficient mice, and revealed HSF1 is required for thermotolerance [22]. However, genome-wide analysis using WT and HSF1-deficient MEFs revealed that HSF1 regulates and induces a lot of genes even under the 37 degrees control condition [30].

Recently, my colleagues and I revealed that HSF1 positively regulates twenty-nine novel target genes and that these genes are non-Hsp [12]. Surprisingly, eight genes − Nuclear Factor of Activated T-cell cytoplasmic 2 (Nfatc2) [13–15], PDZ-domain containing 3 (Pdzk3) [4, 9, 27, 33], alphaB-Crystallin (Cryab) [1, 20], Cysteine and glycine-Rich Protein 2 (Csrp2) [2, 8], Preferentially Expressed Antigen in Melanoma (Prame) [3, 5], Prominin-2 (Prom2) [7, 17], Transmembrane Protease, Serine 3 (Tmpress3) [6, 26], and Dehydrogenase/reductase 2 (Dhrs2) [10, 28] − strongly suppressed polyQ protein aggregations/inclusion [12].

Moreover, in the comparison of WT and HSF1KO MEFs, the Hsp proteins expression levels were almost the same between these two cells. Nevertheless, the expression of Nfatc2, Pdzk3, Cryab, and Csrp2 were prominently reduced in HSF1KO cells. The reduction level of Nfatc2 was especially outstanding.

In the same study, my colleagues and I used R6/2 HD model mice and established HSF1KO-HD mice. We also examined the longevity, neurological symptoms, polyQ protein aggregates/inclusion generation in the striatum and cerebral cortex, and compared all data between WT-HD and HSF1KO-HD mice. In the HSF1KO-HD mice, we found shortened longevity, obvious neurological symptoms, and more aggregates/inclusion generation.

To reveal the molecular mechanism that explains these impressive results, my colleagues and I used a cellular model and discovered that PDZK3 and CRYAB interacts and form an SCF-type E3 complex, and we found that this complex takes hold of proteins and degrades toxic misfolded protein including polyQ. It has been well-known that HSF1 induces chaperone protein Hsps and maintains the protein structure, but our study revealed for the first time that HSF1 not only repair the damaged proteins by Hsps induction but also degrade them using ubiquitin-proteasome system [12]. However, I did not carry out more detailed analysis.

Here, I show the expression levels of these eight genes and polyQ protein aggregates/inclusion generation in each tissue in WT-HD and HSF1KO-HD mice. Through this detailed investigation, I found some unexpected phenomena and conclude the effects of HSF1 deficiency are very broad and complicated.

Materials and Methods

Animal care, use, and establishment of HSF1KO-HD mice

All mice were housed under the controlled lightning room. Lights were on from 8:00 to 20:00 h, 22–25 degrees temperature, 50% humidity, constant ventilation (at least 10 complete fresh-air changes hourly). Food (Certified Pellet Diet MF) was purchased from Oriental Yeast, Japan. Food and water were available *ad libitum*. Five mice were maintained in a single cage. Male and female mice were maintained in different cages.

The original transgenic mouse line R6/2 containing

the human huntingtin gene carrying 154 CAG repeats [21] was obtained from Jackson Laboratory. I purchased 6-week-old male CBA/J and female C57BL/6J inbred mice from Charles River Japan (Atsugi, Kanagawa, Japan). All mice were acclimated for more than 1 week before use in the experiments. I crossed these inbred mice and obtained CBA/J × C57BL/6J F_1 background mice. According to the manual from Jackson Laboratory, I maintained the R6/2 line using these F_1 mice. I maintained R6/2 mice by ovary transplantation for almost ten years. But unexpectedly, I found extended longevity and that the number of CAG repeats was shortened to 95–97 repeats. HSF1KO mice were established and maintained in this laboratory [12].

I crossed these ovary-transplanted female mice with HSF1KO male mice in the same background. By this crossing, I obtained HSF1hetero male R6/2 (CAG95-97) mice. Probably because of CAG shortening, these male mice had a sufficient fertilization ability, thus I crossed them with HSF1 hetero female mice and successfully generated HSF1KO-R6/2 (CAG95-97) mice. In this study, I describe R6/2 (CAG95-97) mice as HD (WT-HD and HSF1KO-HD). For collection of tissues, mice were anesthetized and euthanized with carbon dioxide gas. All experiments related to these mice were reviewed by the Committee for Ethics on Animal Experiments of Yamaguchi University School of Medicine.

Immunofluorescence

Male mouse tissues were dissected and immediately frozen in OCT compound. Sections 10-μm thick were cut out using a CM1900 cryostat (Leica). Immunohistochemistry was performed by using a goat anti-huntingtin polyclonal antibody (N-18, Santa Cruz) and fluorescein isothiocyanate-conjugated rabbit anti-goat IgG (Jackson). The sections were mounted in VECTASHIELD with 4,6-diamidino-2-phenylindole (Vector Laboratories) and examined by Axioplan 2 microscopy (Zeiss).

Semi-quantitative RT-PCR from mouse tissues

Male mouse tissues were dissected and immediately frozen with liquid nitrogen. After that, total RNA was extracted using TRIzol (Invitrogen). Two μg of RNA from every tissue were applied for reverse-transcribed reaction by AMV kit (Invitrogen) using random primers. Synthesized cDNA was applied for PCR by Ex-Taq polymerase (Takara). As an internal control indicating the same amount of RNA was applied, the cDNA of ribosomal RNA S18 was also synthesized. Primer sequences were described previously [12]. After electrophoresis, the quantities of the bands were determined by Image-J provided by NIH (http://imagej.nih.gov/ij/).

Western blot analysis

The tissues were acquired by the same method as for RT-PCR. The tissues were homogenized in NP40 lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP40) with protease inhibitors (PMSF, pepstatin, leupeptin). After centrifugation, the supernatant was collected. One hundred μg of soluble protein was applied to SDS-PAGE (10% acrylamide) and blotted onto nitrocellulose membrane. The membrane was subjected to immunoblotting using anti-polyQ monoclonal antibody (Millipore), and anti-β-actin antibody (Sigma).

Statistical analysis

The statistical significance was determined by Student's *t*-test. In all experiments, I considered the difference was significant when *P* values less than 0.05.

Results

The expression of the non-Hsp HSF1 target genes that prevent toxic protein aggregation is prominently reduced in HSF1KO-HD mouse tissues

In the previous study, my colleagues and I found twenty-nine novel HSF1 target genes, and the eight genes of them have a strong anti-aggregates/inclusion ability [12]. Therefore, I considered it is necessary to analyze the previous experimental results in detail and carried out some additional experiments.

First, I examined whether HSF1 induces PDZK3/CRYAB protein degradation complex in various tissues *in vivo*, or some other novel HSF1 target genes prevent protein aggregation by unknown mechanisms. To understand how HSF1 maintains the homeostasis at the individual animal level, I examined and compared the expression of these eight genes in each tissue of control and HSF1KO-HD mice by RT-PCR.

The result is shown in Fig. 1. I examined ten tissues; brain, heart, muscle, lung, liver, kidney, pancreas, spleen, stomach, and testis. At first, I examined the expression of these eight genes in control mice and found the expression levels of eight genes is very contrasting. Nfatc2, Pdzk3, Cryab, and Csrp2 were expressed in all or most of the tissues including brain, heart, and muscle. Prame

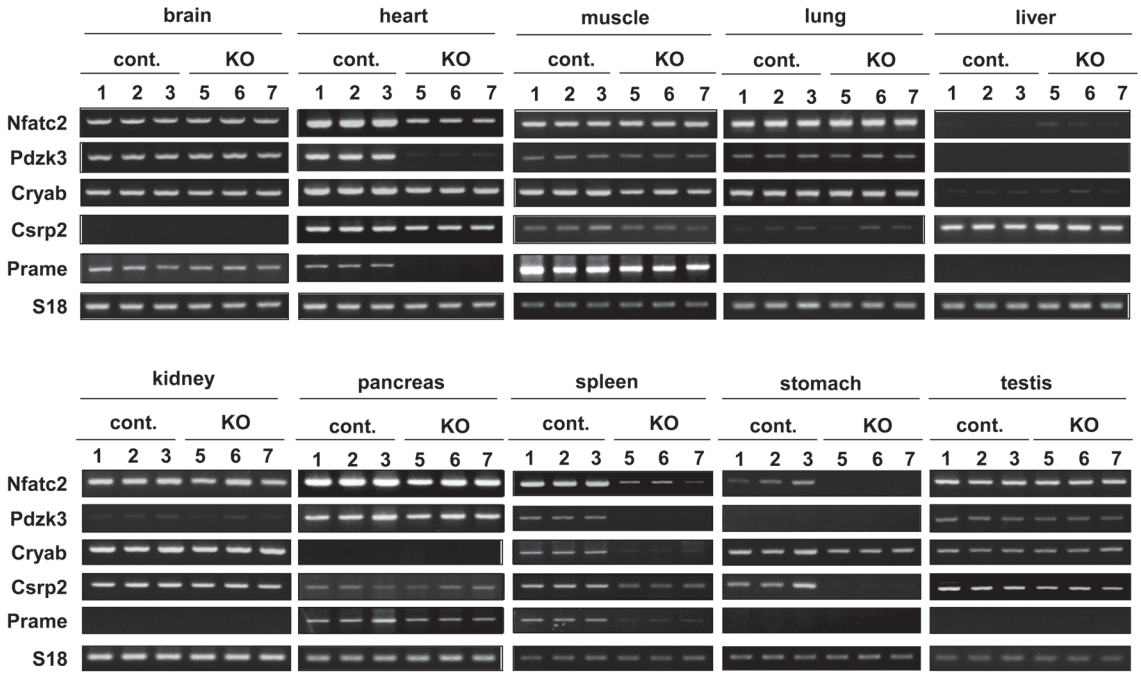

Fig. 1. The expression of anti-aggregative gene mRNA. Eight genes were analyzed, but only five genes, Nfatc2, Pdzk3, Cryab, Csrp2, and Prame, were detected in more than five tissues. Thus the results for only five genes are shown here. The numbers shown above PCR bands (1, 2, 3, 5, 6, and 7) indicate the mouse number. S18 is the internal control indicating that the same amount of RNA was applied.

was expressed in five tissues. In contrast, the other three genes, Prom2, Tmpress3, and Dhrs2 were expressed in only a few tissues. Prom2 was detected in the lung, kidney, and testis. Tmpress3 was in testis, and Dhrs2 was in lung and testis.

Nfatc2 was detected in nine tissues except liver. Pdzk3 was detected in eight tissues, but the level was very low in the kidney. Cryab was detected in nine tissues, but low in the liver. Csrp2 was detected in nine tissues and low in lung. Prame was detected in brain, heart, muscle, pancreas, and spleen.

I compared the expression levels of the eight genes between control and HSF1KO-HD mice in each tissue that I examined. In this analysis, I found that the expression levels of some genes were prominently reduced by HSF1 deficiency but the other genes were slightly affected or not affected at all.

I found dramatic changes in heart, spleen, and stomach (Fig. 1). In heart, prominent reductions of Pdzk3 and Prame expression was occurred. Nfatc2 also showed a marked reduction. Cryab and Csrp2 showed a slight reduction. Thus, the expression levels of these five genes were reduced in the heart. In spleen, the five genes were expressed, but only Nfatc2 showed clear PCR bands, but the other four genes did not show the strong PCR bands even PCR amplification was performed with 40 cycles. But importantly, Pdzk3, Cryab, and Prame were not detected in the HSF1KO-HD mouse spleen. Similarly, Nfatc2 and Csrp2 were also decisively down-regulated in HSF1KO-HD mouse spleen. In the stomach, Nfatc2, Cryab, and Csrp2 were detected but Pdzk3 and Prame were not even in control tissues. However, Nfatc2 and Csrp2 were not detected in HSF1KO-HD stomach. Cryab showed a prominent reduction in the HSF1KO-HD mouse spleen.

Unexpectedly, no change of gene expression by HSF1 deficiency was found in the other seven tissues; brain, muscle, lung, liver, kidney, pancreas, and testis. In addition, the liver was very distinctive, only Csrp2 was detected.

These results indicate that HSF1 regulates these eight important genes in mouse tissues as well as in MEF cells [12]. However, HSF1 does not dominantly regulates in all tissue. Importantly, these genes strongly controlled in the heart, spleen, and stomach.

Higher level of soluble pre-aggregative polyQ-huntingtin protein in HSF1KO-HD mouse tissues

After being maintained by my colleagues for a few

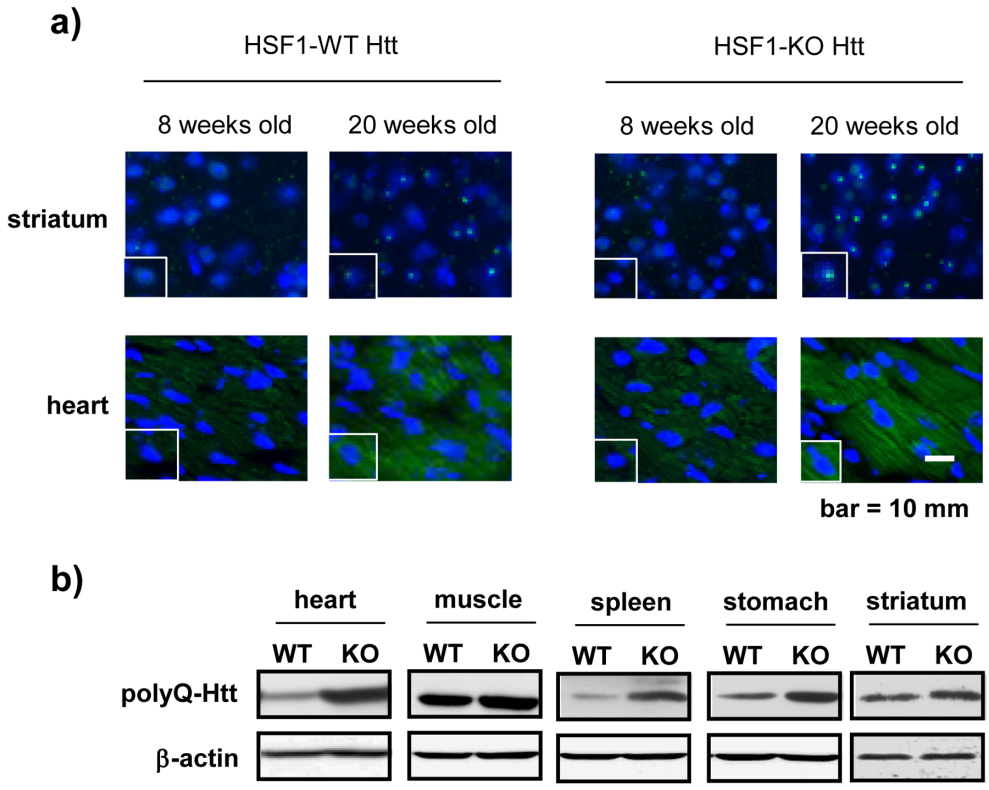

Fig. 2. (a) Immunohistochemical detection of polyQ-huntingtin protein aggregates/inclusions. Aggregate formation was observed in the striatum, but not in the heart. The nuclei are stained by DAPI (blue), and polyQ-huntingtin protein is shown in green by FITC-conjugated secondary antibody. (b) Western blot analysis of polyQ protein in the heart, muscle, spleen, and striatum. The cells inside white boxes in the lower left of each figure are typical cells with aggregates/inclusions.

years, I have similarly maintained HD mice for almost ten years by ovary transplantation. The original HD (R6/2) mice established by Bates and his colleagues have 154 CAG repeats [21]. However, our R6/2 mice had only 95–97 CAG repeats probably caused by repeated ovary transplantation, thus the phenotype of our HD mice has become milder than the original [12]. I established HSF1KO-HD mice, but surprisingly, these mutant HD mice showed the prominently shortened longevity and increased polyQ-huntingtin N-terminal protein (polyQ-HTT) aggregations in striatum and cerebral cortex. Although our HD mice have shorter CAG repeats, the phenotype was similar to that of the original R6/2 mice [12].

Bates and his colleagues also established full-length human huntingtin (containing 150 glutamine tract)-knock-in mice (*Hdh*Q150 knock-in mice) [25]. They used R6/2 and *Hdh*150 knock-in mice, and compared how much polyQ aggregation are formed [25]. As a result, they found typical polyQ inclusion bodies in each tissue they examined in both mouse types. The largest number of polyQ inclusion bodies were found in adrenal gland medulla and pancreas in both types, in contrast, a small number of polyQ inclusion bodies were found in heart cardiac muscle fibers.

Several papers reported that cardiac dysfunction occurs in both Huntington's disease patients and Huntington's disease model mice [19, 23, 24, 29]. Thus, I examined whether polyQ aggregates formed in the heart and other tissues in our HD mice or not.

I examined ten tissues of 8-weeks-old mice. Surprisingly, I could not find any polyQ aggregates/inclusion. In the heart, I could not find any polyQ aggregates or inclusion, but the soluble (pre-aggregative) polyQ protein level was lower in WT-HD mice than HSF1KO-HD (Fig. 2). I found the similar difference in other tissues, including the muscle, spleen, stomach, and also striatum. I examined 20-weeks-old HD mice, but polyQ aggregates/inclusion were not observed in the heart in either type of HD mouse. Our HD mice have a shorter polyQ tract than

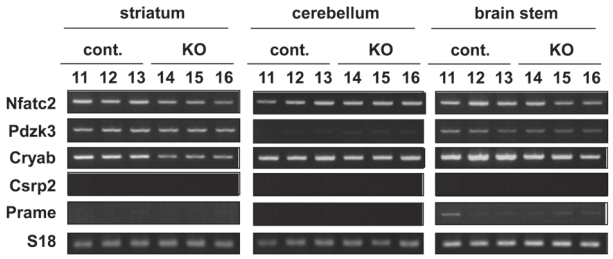

Fig. 3. The expression of five anti-aggregative genes in the brain. The striatum, cerebellum, and brain stem were analyzed by RT-PCR. The numbers shown above PCR bands (11, 12, 13, 14, 15, and 16) indicate the mouse number. S18 is internal control indicating that the same amount of RNA was applied.

the original R6/2, thus our HDs' polyQ-HTT protein probably does not form aggregates/inclusion easily.

Additionally, I examined the brain again after segregating it into three parts, striatum, cerebellum, and brain stem. I found polyQ aggregation again in the striatum, but not in the other two parts. In those two parts, the polyQ protein expression level was almost the same in the control and HSF1KO mice.

Next, I also examined the expression levels of the eight genes in these three parts to analyze the reason why polyQ aggregation appears in only the striatum (Fig. 3). I found that Csrp2 and Prame were not expressed, but other three genes, Nfatc2, Pdzk3, and Cryab were expressed. Prom2, Tmpress3, and Dhrs2 were not detected in all three parts as well as whole brain. In the striatum, I found the reduced expression of these three genes in HSF1KO-HD mice, but not in the cerebellum and brain stem (Fig. 4). As shown in this study and my previous paper [12], polyQ expression and aggregates were found in the striatum more often in the HSF1KO-HD mice more than the control. The mRNA expression levels of Nfatc2, Pdzk3, and Cryab must be related to the polyQ aggregation level because the products of these genes have the ability to degrade protein. However, the reduction in mRNA expression of these three genes in the striatum was less than I expected. In contrast, polyQ protein aggregations/inclusion were not formed in heart and spleen, but their soluble protein level was prominently reduced. Congenital anti-aggregative ability is probably different among these three tissues, but this idea is just one hypothesis.

Expression of Hsps is not prominently changed in HSF1KO-HD mouse tissues

In Fig. 2, I show the five genes, Nfatc2, Pdzk3, Cryab, Csrp2, and Prame are expressed in many tissues compared to other three genes, Prom2, Tmpress3, and Dhrs2. Moreover, the expression levels of the former five genes expression are reduced in some HSF1KO-HD tissues.

However, I did not examined whether heat shock protein (Hsp), the most classical target genes of HSF1, are affected in their expression by HSF1 deficiency. Hsps directly bind to intracellular proteins and maintain the precise folding of denatured proteins, thus it is important whether Hsp expression level is affected in WT-HD and HSF1KO-HD.

Benjamin and his colleagues showed that CRYAB, Hsp25, and Hsp70 are decreased in the HSF1KO mouse (not HD mouse) heart at the protein level but that Hsp60 and Hsp90 are not changed [32]. I examined Hsp70, Hsp40, and Hsp27 expression in the striatum, heart, and spleen by RT-PCR. In the striatum, these three Hsps levels in control mice were almost the same as those in HSF1KO-HD mice. In the heart, only a slight difference was found in Hsp70, and there was no difference in Hsp40 and Hsp27 between control and HSF1KO-HD mice. In the spleen, a clear difference was found in Hsp70 and Hsp40, their expression was decreased to 75% and 60%, respectively. But Hsp27 level was very low and did not show a significant difference (Fig. 5).

I previously discovered accelerated polyQ protein aggregation in the HSF1KO-HD mouse striatum, but the Hsp expression of HSF1KO-HD mice was similar to that of the controls. Similarly, I could not find any prominent difference in the expression levels of Hsps in the heart. The largest difference was found in the spleen, but the percentage was less than 75%.

These results indicate that the change in Hsp expression level caused by HSF1 deficiency is subtle in a variety of tissues, therefore, the novel target genes, especially Nfatc2, Pdzk3, Cryab, Csrp2, and Prame, have more pivotal roles in maintenance of the homeostasis in tissues and organs than Hsps.

Discussion

In this study, I used WT-HD and HSF1KO-HD mice to investigate whether polyQ protein aggregates/inclusion generated in other tissues in addition to the brain. The original R6/2 mice (150CAG) established by Bates

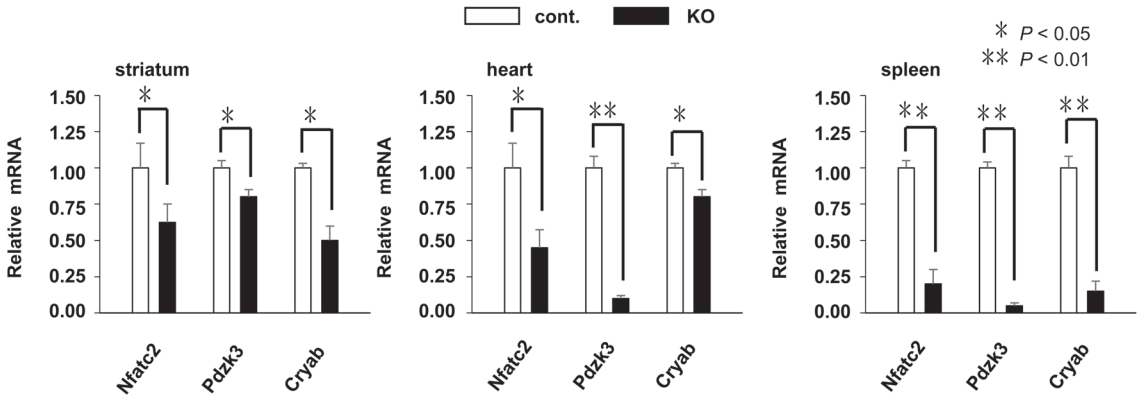

Fig. 4. The expression levels of Nfatc2, Pdzk3, Cryab in the striatum, heart, and spleen.

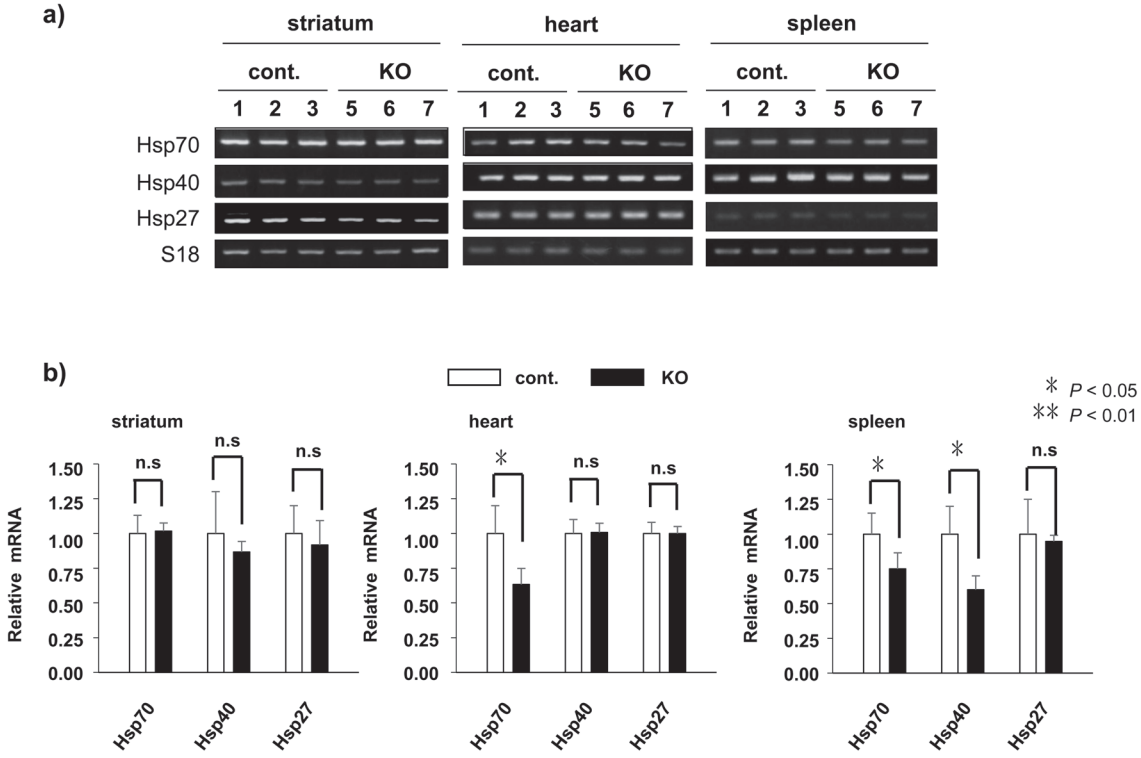

Fig. 5. (a) The expression levels of Hsp70, Hsp40, Hsp27 in the striatum, heart, and spleen. The numbers shown above PCR bands (1, 2, 3, 5, 6, and 7) indicate the mouse number. (b) The expression levels of Hsps shown in (a) were quantified by Image-J. The results are shown as graphs and significance is indicated when the *P*-value is less than 0.05. S18 is the internal control indicating that the same amount of RNA was applied.

group show clear inclusion bodies in all tissues examined [25]. Previously, I examined the aggregates/inclusion in the brain including the striatum and found increased aggregates in the HSF1KO-HD striatum, but they did not easily grow into complete inclusion. Almost 40 weeks were necessary to develop the typical inclusion bodies [12].

In this study, I investigated aggregates/inclusion generation in each tissue examined. The brain was separated into the striatum, cerebellum, brain stem, and re-investigated. Surprisingly, I did not find any aggregates/inclusion generation in any tissues of the WT-HD nor even in HSF1KO-HD mice except in the striatum. However, the soluble pre-aggregative polyQ protein levels in

WT-HD tissues were lower than those in HSF1KO tissues. I also tried to find aggregates in postmortem mouse tissues but failed.

Furthermore, I also examined the expression of eight essential genes and found Nfatc2, Pdzk3, Cryab, and Csrp2 are expressed in each tissue. As I already revealed, NFATc2 up-regulates Pdzk3 and Cryab together with HSF1, and PDZK3 and CRYAB form an SCF-type E3 ubiquitin ligase complex. The PDZK3/CRYAB complex can efficiently degrade polyQ proteins [12]. I recognized that the eight genes are important for intracellular homeostasis, and the fact that Nfatc2, Pdzk3, and Cryab are expressed in many tissues indicates these three genes may be especially important for most tissues and many kinds of cell *in vivo*.

Before I started this research, I expected HSF1 deficiency significantly affects the gene expression at least in several tissues. But actually, prominent effects appeared in only heart, spleen, and stomach. In the heart and spleen, Pdzk3 expression was almost completely diminished by HSF1 deficiency. In the stomach, Pdzk3 was not detected in either WT or HSF1KO-HD mice. In these tissues, Csrp2 was affected by HSF1 deficiency in addition to Nfatc2, Pdzk3, and Cryab.

I found that Csrp2 was expressed in nine tissues in this study, importantly, its strong anti-aggregation ability at the same level to the other three genes were previously revealed [12]. CSRP2 has a LIM zinc finger domain, and this domain is known as protein-protein interaction. CSRP2 may be involved in the PDZK3/CRYAB E3 complex. At the least, CSRP2 may have an anti-aggregation ability, and the ability may not be related to PDZK3/CRYAB E3 complex.

This time, I separated the brain into three regions, striatum, cerebellum, and brain stem. I found polyQ aggregates in only the striatum and an increase of aggregates in the same region of HSF1KO-HD mice. However, the effects of HSF1 deficiency to gene expression were not strong, Nfatc2, Pdzk3, and Cryab showed 50–20% reduction in expression (Fig. 4). Csrp2 was not detected. Because of a lack of credible antibodies, I could not examine the protein levels of NFATc2, PDZK3, and CRYAB. Thus, there is still a possibility that HSF1 deficiency causes a prominent reduction in these three proteins.

On the other hand, I examined how much the expression of Hsps is affected by HSF1 deficiency *in vivo*. I examined the protein levels of Hsp70, Hsp40, and Hsp27 in striatum, cerebellum, and brain stem. However, I could not find any difference. Benjamin and his colleagues showed the levels of major Hsps proteins in brain, heart, liver, and kidney [31]. Similar to my results, the protein levels of the major Hsps − Hsp70, Hsp90alpha, Hsp90beta, Hsc70, Hsp60, and Hsp25 − among WT, HSF1hetero, HSF1KO mice were not reduced by HSF1 deficiency except for Hsp25. Hsp25 showed a 50% reduction in HSF1KO mice. However, these data and my results do not show a substantial change in the expression of Hsps caused by HSF1 deficiency, even in the striatum, but the polyQ aggregation level was increased in the HSF1KO-HD striatum. Considering my data, Hsp expression still requires attention, but the novel HSF1 targets, especially Nfatc2, Pdzk3, Cryab, and Csrp2, must be more critical genes than Hsps for intracellular homeostasis *in vitro* and *in vivo*.

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Reference

1. Balch, W.E., Morimoto, R.I., Dillin, A., and Kelly, J.W. 2008. Adapting proteostasis for disease intervention. *Science* 319: 916–919. [Medline] [CrossRef]
2. Chang, D.F., Belaguli, N.S., Iyer, D., Roberts, W.B., Wu, S.P., Dong, X.R., Marx, J.G., Moore, M.S., Beckerle, M.C., Majesky, M.W., and Schwartz, R.J. 2003. Cysteine-rich LIM-only proteins CRP1 and CRP2 are potent smooth muscle differentiation cofactors. *Dev. Cell* 4: 107–118. [Medline] [CrossRef]
3. Costessi, A., Mahrour, N., Tijchon, E., Stunnenberg, R., Stoel, M.A., Jansen, P.W., Sela, D., Martin-Brown, S., Washburn, M.P., Florens, L., Conaway, J.W., Conaway, R.C., and Stunnenberg, H.G. 2011. The tumour antigen PRAME is a subunit of a Cul2 ubiquitin ligase and associates with active NFY promoters. *EMBO J.* 30: 3786–3798. [Medline] [CrossRef]
4. Deguchi, M., Iizuka, T., Hata, Y., Nishimura, W., Hirao, K., Yao, I., Kawabe, H., and Takai, Y. 2000. PAPIN. A novel multiple PSD-95/Dlg-A/ZO-1 protein interacting with neural plakophilin-related armadillo repeat protein/delta-catenin and p0071. *J. Biol. Chem.* 275: 29875–29880. [Medline] [CrossRef]

5. Epping, M.T., Wang, L., Edel, M.J., Carlée, L., Hernandez, M., and Bernards, R. 2005. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. *Cell* 122: 835–847. [Medline] [CrossRef]
6. Fasquelle, L., Scott, H.S., Lenoir, M., Wang, J., Rebillard, G., Gaboyard, S., Venteo, S., François, F., Mausset-Bonnefont, A.L., Antonarakis, S.E., Neidhart, E., Chabbert, C., Puel, J.L., Guipponi, M., and Delprat, B. 2011. Tmprss3, a transmembrane serine protease deficient in human DFNB8/10 deafness, is critical for cochlear hair cell survival at the onset of hearing. *J. Biol. Chem.* 286: 17383–17397. [Medline] [CrossRef]
7. Florek, M., Bauer, N., Janich, P., Wilsch-Braeuninger, M., Fargeas, C.A., Marzesco, A.M., Ehninger, G., Thiele, C., Huttner, W.B., and Corbeil, D. 2007. Prominin-2 is a cholesterol-binding protein associated with apical and basolateral plasmalemmal protrusions in polarized epithelial cells and released into urine. *Cell Tissue Res.* 328: 31–47. [Medline] [CrossRef]
8. Grubinger, M. and Gimona, M. 2004. CRP2 is an autonomous actin-binding protein. *FEBS Lett.* 557: 88–92. [Medline] [CrossRef]
9. Harris, B.Z. and Lim, W.A. 2001. Mechanism and role of PDZ domains in signaling complex assembly. *J. Cell Sci.* 114: 3219–3231. [Medline]
10. Hawkins, J., Mahony, D., Maetschke, S., Wakabayashi, M., Teasdale, R.D., and Bodén, M. 2007. Identifying novel peroxisomal proteins. *Proteins* 69: 606–616. [Medline] [CrossRef]
11. Hayashida, N., Inouye, S., Fujimoto, M., Tanaka, Y., Izu, H., Takaki, E., Ichikawa, H., Rho, J., and Nakai, A. 2006. A novel HSF1-mediated death pathway that is suppressed by heat shock proteins. *EMBO J.* 25: 4773–4783. [Medline] [CrossRef]
12. Hayashida, N., Fujimoto, M., Tan, K., Prakasam, R., Shinkawa, T., Li, L., Ichikawa, H., Takii, R., and Nakai, A. 2010. Heat shock factor 1 ameliorates proteotoxicity in cooperation with the transcription factor NFAT. *EMBO J.* 29: 3459–3469. [Medline] [CrossRef]
13. Hodge, M.R., Ranger, A.M., Charles de la Brousse, F., Hoey, T., Grusby, M.J., and Glimcher, L.H. 1996. Hyperproliferation and dysregulation of IL-4 expression in NF-ATp-deficient mice. *Immunity* 4: 397–405. [Medline] [CrossRef]
14. Hogan, P.G., Chen, L., Nardone, J., and Rao, A. 2003. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev.* 17: 2205–2232. [Medline] [CrossRef]
15. Horsley, V., Jansen, K.M., Mills, S.T., and Pavlath, G.K. 2003. IL-4 acts as a myoblast recruitment factor during mammalian muscle growth. *Cell* 113: 483–494. [Medline] [CrossRef]
16. Jackson, S.E., Queitsch, C., and Toft, D. 2004. Hsp90: from structure to phenotype. *Nat. Struct. Mol. Biol.* 11: 1152–1155. [Medline] [CrossRef]
17. Jászai, J., Farkas, L.M., Fargeas, C.A., Janich, P., Haase, M., Huttner, W.B., and Corbeil, D. 2010. Prominin-2 is a novel marker of distal tubules and collecting ducts of the human and murine kidney. *Histochem. Cell Biol.* 133: 527–539. [Medline] [CrossRef]
18. Kingston, R.E., Schuetz, T.J., and Larin, Z. 1987. Heat-inducible human factor that binds to a human hsp70 promoter. *Mol. Cell. Biol.* 7: 1530–1534. [Medline]
19. Lanska, D.J., Lavine, L., Lanska, M.J., and Schoenberg, B.S. 1988. Huntington's disease mortality in the United States. *Neurology* 38: 769–772. [Medline] [CrossRef]
20. Lin, D.I., Barbash, O., Kumar, K.G., Weber, J.D., Harper, J.W., Klein-Szanto, A.J., Rustgi, A., Fuchs, S.Y., and Diehl, J.A. 2006. Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(FBX4-alphaB crystallin) complex. *Mol. Cell* 24: 355–366. [Medline] [CrossRef]
21. Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S.W., and Bates, G.P. 1996. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* 87: 493–506. [Medline] [CrossRef]
22. McMillan, D.R., Xiao, X., Shao, L., Graves, K., and Benjamin, I.J. 1998. Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis. *J. Biol. Chem.* 273: 7523–7528. [Medline] [CrossRef]
23. Mielcarek, M., Bondulich, M.K., Inuabasi, L., Franklin, S.A., Muller, T., and Bates, G.P. 2014. The Huntington's disease-related cardiomyopathy prevents a hypertrophic response in the R6/2 mouse model. *PLoS ONE* 9: e108961. [Medline] [CrossRef]
24. Mielcarek, M., Inuabasi, L., Bondulich, M.K., Muller, T., Osborne, G.F., Franklin, S.A., Smith, D.L., Neueder, A., Rosinski, J., Rattray, I., Protti, A., and Bates, G.P. 2014. Dysfunction of the CNS-heart axis in mouse models of Huntington's disease. *PLoS Genet.* 10: e1004550. [Medline] [CrossRef]
25. Moffitt, H., McPhail, G.D., Woodman, B., Hobbs, C., and Bates, G.P. 2009. Formation of polyglutamine inclusions in a wide range of non-CNS tissues in the HdhQ150 knock-in mouse model of Huntington's disease. *PLoS ONE* 4: e8025. [Medline] [CrossRef]
26. Molina, L., Fasquelle, L., Nouvian, R., Salvetat, N., Scott, H.S., Guipponi, M., Molina, F., Puel, J.L., and Delprat, B. 2013. Tmprss3 loss of function impairs cochlear inner hair cell Kcnma1 channel membrane expression. *Hum. Mol. Genet.* 22: 1289–1299. [Medline] [CrossRef]
27. Ohno, H., Hirabayashi, S., Iizuka, T., Ohnishi, H., Fujita, T., and Hata, Y. 2002. Localization of p0071-interacting proteins, plakophilin-related armadillo-repeat protein-interacting protein (PAPIN) and ERBIN, in epithelial cells. *Oncogene* 21: 7042–7049. [Medline] [CrossRef]
28. Shafqat, N., Shafqat, J., Eissner, G., Marschall, H.U., Tryggvason, K., Eriksson, U., Gabrielli, F., Lardy, H., Jörnvall, H., and Oppermann, U. 2006. Hep27, a member of the short-chain dehydrogenase/reductase family, is an NADPH-dependent dicarbonyl reductase expressed in vascular endothelial tissue. *Cell. Mol. Life Sci.* 63: 1205–1213. [Medline] [CrossRef]
29. Sørensen, S.A. and Fenger, K. 1992. Causes of death in patients with Huntington's disease and in unaffected first degree relatives. *J. Med. Genet.* 29: 911–914. [Medline]

[CrossRef]

30. Trinklein, N.D., Murray, J.I., Hartman, S.J., Botstein, D., and Myers, R.M. 2004. The role of heat shock transcription factor 1 in the genome-wide regulation of the mammalian heat shock response. *Mol. Biol. Cell* 15: 1254–1261. [Medline] [CrossRef]
31. Xiao, X., Zuo, X., Davis, A.A., McMillan, D.R., Curry, B.B., Richardson, J.A., and Benjamin, I.J. 1999. HSF1 is required for extra-embryonic development, postnatal growth and protection during inflammatory responses in mice. *EMBO J.* 18: 5943–5952. [Medline] [CrossRef]
32. Yan, L.J., Christians, E.S., Liu, L., Xiao, X., Sohal, R.S., and Benjamin, I.J. 2002. Mouse heat shock transcription factor 1 deficiency alters cardiac redox homeostasis and increases mitochondrial oxidative damage. *EMBO J.* 21: 5164–5172. [Medline] [CrossRef]
33. Yeung, M.L., Tam, T.S., Tsang, A.C., and Yao, K.M. 2003. Proteolytic cleavage of PDZD2 generates a secreted peptide containing two PDZ domains. *EMBO Rep.* 4: 412–418. [Medline] [CrossRef]