Heat shock transcription factor 1 is required for maintenance of ciliary beating in mice

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Heat shock transcription factors (HSFs) maintain protein homeostasis through regulating expression of heat shock proteins, especially in stressed conditions. In addition, HSFs are involved in cellular differentiation and development by regulating development-related genes, as well as heat shock genes. Here, we showed chronic sinusitis and mild hydrocephalus in postnatal HSF1-null mice, which are associated with impaired mucociliary clearance and cerebrospinal flow, respectively. Analysis of ciliary beating revealed that the amplitude of the beating was significantly reduced, and ciliary beat frequencies were lower in the respiratory epithelium, ependymal cells, oviduct, and trachea of HSF1-null mice than those of wild-type mice. Cilia possess a common axoneme structure composed of microtubules of α- and β-tubulin. We found a marked reduction in α- and ciliary βγ-tubulin in the HSF1-null cilia, which is developmentally associated with reduced Hsp90 expression in HSF1-null mice. Treatment of the respiratory epithelium with geldanamycin resulted in rapid reduction of ciliary beating in a dose-dependent manner. Furthermore, Hsp90 was physically associated with ciliary βγ-tubulin, and Hsp90 stabilizes tubulin polymerization in vitro. These results indicate that HSF1 is required to maintain ciliary beating in postnatal mice, probably by regulating constitutive expression of Hsp90 that is important for tubulin polymerization.

Heat shock response is characterized by induction of a set of heat shock proteins (Hsps) 5 and is a fundamental adaptive response in all organisms from bacteria to humans. This response is regulated mostly at the level of transcription by heat shock transcription factors that bind to the heat shock element in eukaryotes (1, 2). Among four HSF family members (HSF1–4), HSF1 plays a key role in heat shock response in mammals, whereas HSF3 does so in avians (3, 4). This HSF-mediated induction of Hsps is required for acquisition of thermotolerance (5–7) and protection of cells from various pathophysiological conditions (8–11). Inversely, HSF1 also regulates proapoptotic genes to decide on cell death or life in response to stress (12–14). In addition to the role in heat shock response, HSFs play critical functions in developmental processes such as gametogenesis and neurogenesis (15–20), in maintenance of the sensory organs (21–24), and in immune response (25, 26).

Although the precise mechanisms of how HSFs act in these physiological processes are still unclear, genetic evidence shows that HSFs regulate constitutive gene expression in unstressed cells and tissues (27, 28). Furthermore, it was revealed in sensory and immune cells that HSFs not only maintain protein homeostasis by regulating constitutive expression of Hsps, but are also involved in cell growth and differentiation by regulating expression of cytokines such as interleukin-6, fibroblast growth factors, and LIF (22, 24, 25). However, we do not know any client protein stabilized by Hsps that is under the control of HSFs in unstressed conditions.

We previously showed abnormal nasal cavities in HSF1-null adult mice, which is associated with atrophy of the olfactory epithelium and sinusitis characterized by accumulation of mucus (24), and demonstrated that HSF1 is required to maintain olfactory neurogenesis. However, the mucus accumulation cannot be explained by this function of HSF1. Here, we examined the molecular mechanisms underlying mucus accumulation in the HSF1-null nasal cavity, and demonstrated that HSF1 is required to maintain ciliary beating in many organs, probably by regulating constitutive expression of Hsp90 that facilitates tubulin polymerization. This is the first demonstration that HSF1 plays a role in maintaining dynamic movement of a microstructure in cells.

MATERIALS AND METHODS

Histopathology and Immunohistochemistry—HSF1-null (7) mice were maintained by crossing with ICR mice. Mice were
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systemically anesthetized with ketamine (16 mg/kg, intraperitoneal) and xylazine (16 mg/kg, intraperitoneal), and transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). Tissues were removed and soaked in 4% paraformaldehyde for 12 h at 4 °C. After washing with PBS, the blocks were dehydrated, embedded in paraffin, and cut into sections 5-μm thick. To prepare nasal sections, the fixed tissues were incubated in K-CX decalcification solution (Fujisawa, Osaka, Japan) for 24 h, and then neutralized in 5% Na2SO4 for 24 h. The sections were stained with hematoxylin and eosin. To identify mucus, sections were stained with periodic acid-Schiff.

Immunostaining of the paraffin sections was performed as described previously (12). Antibodies used were antisera for mouse Hsp110 (αHsp110α), human Hsp90 (αHsp90c), human Hsp70 (αHsp70-1), human Hsp40 (αHsp40-1) (24, 29), mouse Hsp27 (αmHsp27c), mouse TCP-1α (αmTCP-1α) (both were generated by immunizing rabbits with recombinant mouse Hsp27 or TCP-1α), DH2 and LIC3 (30), or monoclonal antibodies for α-tubulin (fluorescein isothiocyanate-conjugated mouse IgG, F2168, Sigma) and βtubulin (T7941, Sigma). Peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG was used as a second antibody. Signals were detected using a diaminobenzidine-conjugated goat anti-rabbit IgG (DAB substrate kit (Vector Laboratories, Inc.). Sections were counterstained with methyl green. To perform double staining, the sections were incubated in K-CX decalcification solution (Fujisawa, Osaka, Japan) for 24 h, and then neutralized in 5% Na2SO4 for 24 h. The sections were stained with hematoxylin and eosin. To identify mucus, sections were stained with periodic acid-Schiff and counterstained with hematoxylin.

Estimation of Mucociliary Clearance—Analysis of the nasal mucociliary clearance was performed as described previously (12). Antibodies used were antisera for mouse Hsp110 (αHsp110α), human Hsp90 (αHsp90c), human Hsp70 (αHsp70-1), human Hsp40 (αHsp40-1) (24, 29), mouse Hsp27 (αmHsp27c), mouse TCP-1α (αmTCP-1α) (both were generated by immunizing rabbits with recombinant mouse Hsp27 or TCP-1α), DH2 and LIC3 (30), or monoclonal antibodies for α-tubulin (fluorescein isothiocyanate-conjugated mouse IgG, F2168, Sigma) and βtubulin (T7941, Sigma). Peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG was used as a second antibody. Signals were detected using a DAB substrate kit (Vector Laboratories, Inc.). Sections were counterstained with methyl green. To perform double staining, the sections were incubated in K-CX decalcification solution (Fujisawa, Osaka, Japan) for 24 h, and then neutralized in 5% Na2SO4 for 24 h. The sections were stained with hematoxylin and eosin. To identify mucus, sections were stained with periodic acid-Schiff and counterstained with hematoxylin. Immunostaining of the paraffin sections was performed as described previously (12). Antibodies used were antisera for mouse Hsp110 (αHsp110α), human Hsp90 (αHsp90c), human Hsp70 (αHsp70-1), human Hsp40 (αHsp40-1) (24, 29), mouse Hsp27 (αmHsp27c), mouse TCP-1α (αmTCP-1α) (both were generated by immunizing rabbits with recombinant mouse Hsp27 or TCP-1α), DH2 and LIC3 (30), or monoclonal antibodies for α-tubulin (fluorescein isothiocyanate-conjugated mouse IgG, F2168, Sigma) and βtubulin (T7941, Sigma). Peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG was used as a second antibody. Signals were detected using a diaminobenzidine-conjugated goat anti-rabbit IgG (DAB substrate kit (Vector Laboratories, Inc.). Sections were counterstained with methyl green. To perform double staining, the sections were incubated in K-CX decalcification solution (Fujisawa, Osaka, Japan) for 24 h, and then neutralized in 5% Na2SO4 for 24 h. The sections were stained with hematoxylin and eosin. To identify mucus, sections were stained with periodic acid-Schiff and counterstained with hematoxylin.
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RESULTS

Mucus Accumulation in the Nasal Cavity and Hydrocephalus in HSF1-null Mice—Previously, we showed abnormal nasal cavity in HSF1-null adult mice, which is associated with atrophy of the olfactory epithelium and the accumulation of mucus (Fig. 1A, a and d) (24). This revealed that HSF1 is required for maintenance of olfactory neurogenesis. However, the mucus accumulation cannot be explained by this function of HSF1. Furthermore, the accumulation of mucus was not ameliorated in mice deficient for both HSF1 and HSF4, whereas the atrophied olfactory epithelium was partially restored in the same mice (24). We examined the mucociliary clearance in the nasal cavity by monitoring movement of Pelican ink (31). The ink injected into the nasal vestibules was cleared after 1 h, and moved to the posterior turbinates (Fig. 1A, b and c). In contrast, the ink was hardly removed from the vestibules of HSF1-null mice even after 3 h (Fig. 1A, e and f), indicating that the mucociliary clearance was severely impaired in HSF1-null mice. Therefore, HSF1 may have a direct role in mucociliary clearance in the nasal cavity.

The whipping movement of the cilia generates moving force of the mucus in the respiratory epithelium. As cilia are present in many organs in the body, any dysfunction caused by genetic

mucus or antiserum for Hsp90 (αHsp90c) was added at 4 °C overnight, and mixed with 40 μl of protein A-Sepharose beads (1:1 suspension in PBS) (Amersham Biosciences) by rotating at 4 °C for 1 h in the presence or absence of 1 μM geldanamycin. The complexes were washed five times with RIPA buffer, suspended in SDS sample buffer, and boiled for 3 min. The samples were loaded on SDS-PAGE, and transferred onto nitrocellulose membranes. The membranes were immunoblotted using a mouse monoclonal antibody for GFP (GF200, Nacalai Tesque, Kyoto, Japan). Alternatively, immunoprecipitation was performed using antibody for GFP, and immunoblot analysis was performed using antiserum for Hsp90.

In Vitro Tubulin Polymerization Assay—Hsp90 was purified from mouse L5178Y cells as described previously (37), and stored in a buffer containing 50 mM Tris-HCl, 100 mM NaCl, 2 mM EDTA, 1 mM dithiothreitol, 10% glycerol at −80 °C until use. A tubulin polymerization assay was performed according to the manufacturer's instructions for a fluorescence-based tubulin polymerization assay kit (BK011, Cytoskeleton, Inc.). Briefly, a tubulin reaction mixture (50 μl) containing tubulin and a fluorescent reporter was mixed with bovine serum albumin (BSA, Fraction V, Nacalai Tesque), Hsp90 (5 μl), or paclitaxel that promoted microtubule polymerization and stability (38) and incubated at 37 °C until 40 min. Fluorescence emission at 460 nm (excitation wavelength is 360 nm) was measured by using a CytoFluor II Fluorescence Multi-well Plate Reader (PerSeptive Biosystems, Inc.).

Statistical Analysis—Significant values were determined by analyzing data with the Mann-Whitney’s U test using StatView version 4.5J for Macintosh (Abacus Concepts, Berkeley, CA). A level of p < 0.05 was considered significant.

FIGURE 1. Mucus accumulation in the nasal cavity and hydrocephalus in HSF1-null mice. A, histological examination of the paranasal sections of 6-week-old wild-type (+/−) (a) and HSF1-null (−/−) (d) mice. Periodic acid-Schiff staining was performed. Arrows indicate mucus, which contains detached olfactory epithelial cells. Se, nasal septum; Tu, Turbinates. Bar, 1 mm. Mucociliary clearance was estimated (b, c, e, and f). Pelican ink was injected into the nasal vestibules (indicated as circles), and mucus transport of the ink was examined at 0 (b), 1 h (c and e), and 3 h (f) after injection in wild-type (b and c) and HSF1-null (e and f) mice (n = 5; 5 individuals). Arrows indicate the turbinates. B, histological examination of the brain of 6-week-old mice by HE staining. Coronal sections are shown. LV, lateral ventricle; 3V, third ventricle; Aq, aqueduct; 4V, fourth ventricle. Bar, 1 mm. C, epidymal flow on the wall of the lateral ventricle. Pelican ink was placed onto the surface of the wall, and its location was shown for 12 s after the injection (n = 5). Dashed red lines denote the lateral ventricle. Red arrows indicate normal direction of the flow. Asterisks indicate the site of wall adhesion. Bar, 1 mm.
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A

B

FIGURE 2. Ciliary dyskinesia in many organs of HSF1-null mice. A, estimation of CBF. Video differential interference contrast images of the respiratory epithelium (nasal), lateral ventricle (brain), trachea, and oviduct of 5-week-old mice in a slice preparation were monitored. CBFs were estimated from frame images (n = 3). Stars indicate p < 0.05. B, transmission electron microscopic analysis of cilia in the respiratory epithelium of wild-type (+/+) (a) and HSF1-null (−/−) (b–d) mice. Cilia with eight microtubule doublets (b), with increased numbers of central microtubules (c), and with translocation of central microtubules (d) are shown. Bar, 100 nm. Percentages of cilia possessing abnormal structures were estimated (n = 3). A star indicates p < 0.05.

mutations of their components cause many phenotypes known as immotile ciliary syndrome or primary ciliary dyskinesia (39, 40). Therefore, we examined the morphology of other organs such as the tracheae, oviducts, and ventricles. Histological examination showed grossly normal morphology in the tracheae and oviducts in 6-week-old HSF1-null mice. However, we noticed hydrocephalus in HSF1-null 6-week-old mice (Fig. 1B). All of the ventricles, including the aqueduct and fourth ventricle, were enlarged, indicating communicating hydrocephalus. Hydrocephalus was observed even in 3-week-old HSF1-null mice, but not in 2-week-old mice (data not shown). Degeneration of neural cells was not found in the brains of 6-week-old HSF1-null mice (data not shown) (20). One possible reason for this phenotype may be impaired flow of cerebrospinal fluid (41). Therefore, we examined the ependymal flow in isolated ventricles by dropping a small amount of Pelican ink onto the exposed surfaces of dissected walls of the lateral ventricles (Fig. 1C). The ink moved along the expected cerebrospinal fluid flow on the wall in the wild-type mice, but stayed in the same place and hardly moved in HSF1-null mice. These results indicate that the ependymal flow is impaired in HSF1-null mice, and this is consistent with the notion of ciliary dysfunction in both the respiratory epithelium and ependymal cells.

Ciliary Beating Is Severely Impaired in HSF1-null Mice—To examine whether or not ciliary movement is impaired in HSF1-null mice, we monitored ciliary beating in the freshly isolated respiratory epithelium of the nasal cavity. The differential interference contrast image showed beating cilia located on the surface of respiratory cells, and each cilium movement was detected by video (supplementary Movies 1 and 2) (33, 34). In wild-type mice, the whipping movement of the cilia was observed and CBF was 13.27 ± 1.53 Hz (Fig. 2A). In marked contrast, the beating amplitude was significantly low, and CBF reduced to 8.70 ± 1.72 Hz in HSF1-null mice. Similarly, CBF in the ventricles of wild-type mice was 18.65 ± 2.54 Hz, whereas that of HSF1-null mice was 8.60 ± 1.82 Hz. Interestingly, the whipping movement of the cilia was markedly impaired even in the tracheae and oviducts (data not shown), and CBF was decreased from 14.38 ± 0.90 and 10.90 ± 0.96 Hz to 11.50 ± 1.82 and 7.52 ± 0.32 Hz in the HSF1-null tracheae and oviducts, respectively (Fig. 2A). These results indicate that HSF1 deficiency causes severe impairment of ciliary beating in many organs.

As the cilium is composed of an axoneme structure that normally consists of nine peripheral microtubule doublets arranged around two central microtubules (39), we next examined the ultrastructure of the cilium by electron microscopy. In the HSF1-null respiratory epithelium, dynein arms and radial spokes were observed, but almost 10% of the cilium possessed abnormal central microtubules and microtubule doublets such as deletion or transposition (Fig. 2B). These abnormalities were characteristic of some types of ciliary dyskinesia (42). Although it is unclear whether the abnormal structures in HSF1-null mice may be responsible for ciliary dyskinesia, these results suggest that assembly or organization of microtubules might be impaired.

Tubulin Expression Decreases in the Motile Cilia of HSF1-null Mice—As HSF1-null mice have abnormal microtubule ultrastructure, we examined expression of their major components such as tubulin, dynein, and chaperonin. Microtubules are formed by protofilaments of α/β-tubulin heterodimers. Among five β-tubulin isotypes, β1 and β2-tubulin are found in all axoneme structures (43, 44), and are required for ciliary function and assembly (45). We found that the level of β4-tubulin in the cilia, as well as ciliary α-tubulin, was significantly decreased in the HSF1-null respiratory epithelium (Fig. 3A). Levels of β4-tubulin were also reduced in cilia of the ventricles, trachea, and oviducts (Fig. 3B). Although the TCP-1 complex, known as eukaryotic cytoplasmic chaperonin, directs folding of cytoskeletal proteins such as α/β/γ-tubulin (46), expression of a component, TCP-1α, was constant in the cilia of HSF1-null mice (Fig. 3A). Furthermore, levels of motor proteins such as dynein heavy chain 2 (DH2) and dynein light intermediate chain 3 (LIC3), which are found in the cilia (30), were also constant in the HSF1-null respiratory epithelium. These data indicate that α- and β4-tubulin are specifically reduced in the cilia of the HSF1-null epithelium.

To examine whether HSF1 directly regulates expression of α- and β4-tubulin genes, we analyzed expression of mRNA levels by using reverse transcriptase-PCR. mRNA levels of α- and β4-tubulin in the HSF1-null respiratory epithelium were the same as those in the wild-type epithelium (Fig. 3C), suggesting that proteins of α- and β4-tubulin may be unstable in the cilia of the HSF1-null epithelium.

Reduction of Hsp90 in Affected Cilia of HSF1-null Mice—To clarify the molecular mechanisms that connect HSF1 defi-
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A

FIGURE 3. Tubulin expression decreases in the cilia of HSF1-null mice. A, immunohistochemical examination of expression levels of major components in the cilia of the respiratory epithelium in 6-week-old wild-type (+/+) and HSF1-null (−/−) mice. The respiratory epithelium was stained with antibody specific for βIV-tubulin, α-tubulin, dynein heavy chain 2 (DH2), dynein light intermediate chain 3 (LIC3), or TCP-1α, and counterstained with methyl green. B, immunohistochemical examination of βIV-tubulin in the cilia of the lateral ventricle (brain), trachea, and oviduct in 6-week-old mice. Bar, 10 μm. C, mRNA levels of tubulins. Reverse transcriptase-PCR analysis of α-, βIV-, and βα-tubulin, as well as S16 ribosomal protein was performed by using total RNAs isolated from the brain, nasal cavity, trachea, and lungs.

C

D

E

FIGURE 4. Reduction of Hsp90 in the cilia of HSF1-null mice. A, immunohistochemical examination of major Hsps in the cilia of the nasal respiratory epithelium in 6-week-old wild-type (+/+) and HSF1-null (−/−) mice. Bar, 10 μm. B, immunohistochemical examination of Hsp90 and Hsp27 in the cilia of the tracheal epithelium, oviduct epithelium, ependymal cells, as well as the nasal respiratory epithelium. C, immunohistochemical examination of Hsp90 and βIV-tubulin in the nasal respiratory epithelium of 3- and 4-week-old mice. D, Western blot analysis of HSF1, Hsp90, and β-actin in the ciliated tissues. Whole tissue extracts isolated from the nasal cavity, trachea, oviducts, and brain were subjected to Western blot analysis. E, chromatin immunoprecipitation-enriched DNAs from tissues of 6-week-old wild-type (+/+) and HSF1-null mice (−/−) using preimmune (P) and anti-HSF1 serum (α-HSF1) as well as input DNA were amplified using primers specific for the Hsp90 promoter (−209 to +56) by PCR analysis.

Decrement with instability of ciliary tubulin, we examined expression of Hsps, products of major HSF1-target genes. In addition to the TCP-1 complex, Hsps such as Hsp70, Hsp90, and Hsp40 are distributed in the cilia and are related to axonemal protein turnover (47–50). Immunohistochemical study showed that major cytoplasmic Hsps such as Hsp110, Hsp90, Hsp70, Hsp40, and Hsp27 were localized both in the cilia and in the cytoplasm of the nasal respiratory epithelium (Fig. 4A). Expression of Hsp110, Hsp70, and Hsp40 did not decrease in the HSF1-null cilia. In marked contrast, Hsp90 and Hsp27 expression significantly decreased in the HSF1-null cilia. Interestingly, Hsp27 expression disappeared both in the cilia and in the cytoplasm, whereas Hsp90 expression disappeared especially in the cilia. Analysis of their expression in other ciliated tissues showed that Hsp27 was less expressed in the tracheal epithelium, oviduct epithelium, and ependymal cells, whereas Hsp90 was highly expressed in all of the epithelia (Fig. 4B). Hsp90 expression markedly decreased in the HSF1-null cilia of the three tissues, as in the respiratory epithelium. Furthermore, Hsp90 expression in the respiratory cilia increased at 4 weeks, but HSF1 deficiency caused decreased expression of both Hsp90 and βIV-tubulin at 4 weeks (Fig. 4C). Thus, decreased Hsp90 expression in the HSF1-null respiratory epithelium is temporally associated with reduction of βIV-tubulin expression. These results strongly suggest the involvement of Hsp90 in the stability of ciliary tubulin.

Although we showed decreased expression of Hsp90 in HSF1-null ciliated epithelial cells by using immunohistochemical analysis, we hardly detected any difference in Hsp90 expression in wild-type and HSF1-null ciliated tissues by Western blot analysis (Fig. 4D). This result may be due to the small numbers of ciliated epithelial cells among total cell numbers of each tissue. Nevertheless, chromatin immunoprecipitation analysis showed that HSF1 bound to a promoter region of the Hsp90α gene (mouse Hsp86 gene) in all four tissues (Fig. 4E). These results strongly suggest that HSF1 directly regulates Hsp90 expression in ciliated epithelial cells.

The Hsp90 Inhibitor Reduces Ciliary Beating—To examine whether Hsp90 is required for ciliary beating, sliced tracheal tissues were soaked with a control solution, and then with a solution containing 1 to 100 nM geldanamycin, a specific inhib-
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![Graph A](image1)

**FIGURE 5.** Hsp90 is required for ciliary beating. A, CBF of the tracheal epithelium of 6-week-old wild-type (left) and HSF1-null (right) mice was estimated in the presence of geldanamycin (GA) at the indicated concentrations (n = 3). CBF ratios (CBF/CBF0, CBF0 is CBF at 0 min) are shown. Stars indicate p < 0.05. B, localization of Hsp90 and βv-tubulin in wild-type and HSF1-null nasal respiratory epithelium. Hsp90 and βv-tubulin were double stained and counterstained with 4',6-diamidino-2-phenylindole. Yellow bars indicate layers of the cilia. White bar, 10 μm. C, effects of geldanamycin on localization of Hsp90 and βv-tubulin in wild-type nasal respiratory epithelium. The slices were treated with 100 nM geldanamycin for 30 min, and then double stained as described above. Bar, 10 μm.

A study showed that Hsp90 directly interacts with ciliary tubulin. As a previous report showed that Hsp90 interacts with purified cytoplasmic microtubules (51), we generated expression vectors for ciliary βv-tubulin and cytoplasmic βii-tubulin fused to GFP (14). We found that Hsp90 interacted with both ciliary βv-tubulin and cytoplasmic βii-tubulin, and this interaction was disrupted in the presence of geldanamycin (Fig. 6A). Hsp90 did not interact with GFP (data not shown). These results demonstrate direct binding of Hsp90 to β-tubulins.

**DISCUSSION**

Epithelial cell cilia possess an axonemal architecture composed of a 9 + 2 arrangement of microtubules composed of α- and β-tubulin, and beat rapidly. This beating moves the fluid across the epithelium, and plays a fundamental role in the mucociliary clearance, left-right patterning (39), generating ependymal flow (41), and neuronal cell migration (32). Reduced movement of ciliary beating causes primary ciliary dyskinesia, including Kartagener syndrome, characterized by situs inversus, bronchiectasis, and chronic sinusitis (40). Such dysmotility or immotility of the cilia results from genetic mutations of major components of the axonema such as dyneins (52–54), DNA polymerase λ (55), sperm-associated antigen 6 (56), and a thioredoxin family member (57). In this study, we demonstrated that HSF1 is required for maintenance of ciliary beating in various organs such as the nasal cavity, ventricles, trachea, and oviducts. Although a reduction in ciliary beating led by HSF1 deficiency did not cause clear phenotypes in all of the...
GFP InputIP:

concentrations of Hsp90 or BSA. Geldanamycin (2 μM) was mixed in some reactions. Fluorescence was detected, and mean ± S.D. are shown (n = 3).

Hsp90 interacts with βi-tubulin and stabilizes tubulin polymerization. A, cell extracts were prepared from 293 cells transfected with an expression vector for GFP-βi-tubulin (β4) or GFP-βi-tubulin (β3), and immunoprecipitation was performed using preimmune (PI) serum, αHsp90, or αGFP in the presence or absence of 1 μM geldanamycin. Co-precipitation of Hsp90 or GFP-fused tubulins was analyzed by Western blotting using each antibody. B, purified mouse Hsp90 and BSA, as well as proteins for molecular mass standards (kDa), were subjected to SDS-PAGE and stained with Coomassie Brilliant Blue. C, time-dependent tubulin polymerization in the presence of Hsp90. A tubulin polymerization assay was performed for the indicated periods at 37 °C without or with Hsp90 (0.39 mg/ml), BSA (0.39 mg/ml), paclitaxel (3 μM), or CaCl2 (25 mM) as a negative control. Fluorescence was detected, and mean ± S.D. are shown (n = 3). D, concentration-dependent tubulin polymerization. Tubulin polymerization assay was performed for 20 min at 37 °C with the indicated concentrations of Hsp90 or BSA. Geldanamycin (2 μM) was mixed in some reactions. Fluorescence was detected, and mean ± S.D. are shown (n = 3).

ciliated tissues, we found chronic sinusitis and mild hydrocephalus in HSF1-null mice.

Previous reports showed that Hsp70, Hsp90, and TCP-1 (CCT) are associated with the axonema in cilia or flagella of various organisms such as chlamydomonas, sea urchins, tetrathymena, and rabbits (47, 58, 59). Furthermore, the folding and assembly of tubulin is facilitated by Hsps such as Hsp70, Hsp90, and TCP-1 (46), and Hsp40, a component of the radial spoke complex (48, 49), is induced during ciliogenesis (60). These observations suggest that Hsps are required for formation and function of cilia. Among these molecular chaperones, we found that Hsp90 expression is specifically regulated by HSF1 in the cilia of various tissues in unstressed postnatal mice, and induced during postnatal development via an HSF1-dependent pathway (Fig. 4). As reduction of Hsp90 expression by HSF1 deficiency is significant, we expect that it could be a cause of ciliary dysfunction. A previous report shows that fruit flies carrying an Hsp90 mutant possess abnormal axonema structure in spermatids, and Hsp90 function is connected to microtubule dynamics (51). As abnormal axoneme structure is detected only in 10% of HSF1-null cilia (Fig. 2), it could not be a cause of ciliary dyskinesia. However, it suggests that assembly or organization of microtubules may be impaired. In fact, treatment of respiratory tissues with geldanamycin resulted in rapid reduction of ciliary beating in a dose-dependent manner (Fig. 5). Although it is unclear whether reduced tubulin levels in HSF1-null cilia are related with ciliary dysfunction, Hsp90 does promote tubulin polymerization in vitro (Fig. 6). These results strongly suggest that HSF1-Hsp90 regulation may be required for ciliary beating under physiological conditions.

In chicken B lymphocytes, both HSF1 and HSF3 specifically up-regulate the expression of Hsp90α under normal growth conditions. This regulation is required at moderately high temperatures to stabilize the Cdc2 protein that promotes cell cycle transition from the G2 phase to the mitosis phase, but is not required in unstressed conditions (27). Disruption of mouse HSF genes revealed tissue-specific regulation of basal expression of Hsps during development. HSF1 is activated and induces a set of Hsps during postnatal development of olfactory neurogenesis in mice (24). In the adult mouse heart, HSF1 is required for basal expression of small Hsps that protect against oxidative stress (28). In the lens of postnatal mice, HSF4 regulates expression of Hsp25 and γ-crystallin that has a role in protein stabilization under dehydrated conditions (22, 23). However, we do not know any client protein of these molecular chaperones that explains the phenotypes of the mutant mice. Here, we demonstrated that Hsp90 does promote tubulin polymerization by direct binding. We showed the first client protein whose polymerization could be regulated by HSF1-mediated expression of a molecular chaperone during development.

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REFERENCES
1. Wu, C. (1995) Annu. Rev. Cell Biol. 11, 441–469
2. Morimoto, R. I. (1998) Genes Dev. 12, 3788–3796
3. Nakai, A. (1999) Cell Stress Chaperones 4, 86–93
4. Pirkkala, L., Nykanen, N., and Sistonen, L. (2001) FASEB J. 15, 1118–1131
5. Takabe, M., Kawazoe, Y., Takeda, S., Morimoto, R. I., Nagata, K., and Nakai, A. (1998) EMBO J. 17, 1750–1758
6. McMillan, D. R., Xiao, X., Shao, L., Graves, K., and Benjamin, I. I. (1998) J. Biol. Chem. 273, 7523–7528
7. Inouye, S., Katsuki, K., Izu, H., Fujimoto, M., Sugahara, K., Yamada, S., Shinkai, Y., Oka, Y., Katoh, Y., and Nakai, A. (2003) Mol. Cell. Biol. 23, 5882–5895
8. Zou, Y., Zhu, W., Sakamoto, M., Qin, Y., Akazawa, H., Toko, H., Mizukami, M., Takeda, N., Minamino, T., Takano, H., Nagai, T., Nakai, A., and Komuro, I. (2003) Circulation 108, 3024–3030
9. Wirth, D., Christians, E., Li, X., Benjamin, I. J., and Gustin, P. (2003) Toxi-
