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HSP90β prevents aging-related cataract formation through regulation of the charged multivesicular body protein (CHMP4B) and p53

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Cataract is a leading ocular disease causing global blindness. The mechanism of cataractogenesis has not been well defined. Here, we demonstrate that the heat shock protein 90β (HSP90β) plays a fundamental role in suppressing cataractogenesis. HSP90β is the most dominant HSP in normal lens, and its constitutive high level of expression is largely derived from regulation by Sp1 family transcription factors. More importantly, HSP90β is significantly down-regulated in human cataract patients and in aging mouse lenses, whereas HSP90β silencing in zebrafish causes cataractogenesis, which can only be rescued by itself but not other HSP90 genes. Mechanistically, HSP90β can directly interact with CHMP4B, a newly-found client protein involved in control of cytokinesis. HSP90β silencing causes upregulation of CHMP4B and another client protein, the tumor suppressor p53. CHMP4B upregulation or overexpression induces excessive division of lens epithelial cells without proper differentiation. As a result, these cells were triggered to undergo apoptosis due to activation of the p53/Bak–Bim pathway, leading to cataractogenesis and microphthalmia. Silence of both HSP90β and CHMP4B restored normal phenotype of zebrafish eye. Together, our results reveal that HSP90β is a critical inhibitor of cataractogenesis through negative regulation of CHMP4B and the p53-Bak/Bim pathway.

lens | cataract | HSP90β | CHMP4B | p53

Cataract is leading ocular disease that causes blindness over the world (1). It is derived from aging, environmental stress, or genetic mutations (2–8). During aging, down-regulation, aggregation, or gradual loss of normal functions of the critical proteins due to oxidation or other modifications will disrupt lens fiber structure and functions to cause cataract (2–7). Besides inducing the above aging changes, environmental stress factors can also trigger apoptosis of lens epithelial cells, and thus interrupt the protection and the repair function of the lens epithelial cells, leading to cataractogenesis (9–12). Genetic mutations, on the other hand, may cause cataract in different mechanisms. So far mutations in more than 60 loci have been shown linked with cataract formation (8). Although how these genes cause cataract remain largely unknown (8), the functional mechanisms of the small heat shock proteins (HSPs), α-crystallins, have been extensively studied (13). Besides acting as lens structure proteins, α-crystallins have chaperone-like activity (14) and are also important antiapoptotic regulators (15). We have previously demonstrated that they can suppress stress-induced apoptosis of lens epithelial cells and subsequent cataract formation through different mechanisms (16–18).

Although the small HSPs, α-crystallins, can act as molecular chaperones to bind damaged proteins in lens, lack of ATPase activity limits their functions. They are not able to recycle the bound protein aggregates derived from aging, environmental stress or gene mutations into cytoplasm (8). In contrast, the heat shock protein 90 (HSP90) can consume ATP to correct misfolding of the client proteins to avoid formation of large protein aggregates and thus is a much better molecular chaperone (19, 20). Indeed, previous studies have well established that HSP90 is the most important chaperone and plays a fundamental role in maintaining the cellular homeostasis (19, 20). By transiently binding its client proteins to protect them from misfolding and aggregation, HSP90 participates in regulation of protein quality control and traffic, signal transduction, cell cycle regulation, apoptosis, gene silencing, and genome maintenance (19, 20). Moreover, changes in HSP90 activity are implicated in pathogenesis of several human diseases including neurodegenerative diseases, cancers, and viral and protozoan infection-related diseases (21–24). Whether HSP90 also plays a role in controlling cataractogenesis remains elusive.

Significance

Cataract is derived from protein aggregation due to genetic mutations, stress and aging. The damaged protein aggregates are bound by α-crystallins but cannot be recycled into cytoplasm due to their functional limitations. Thus, maintaining lens transparency requires functions of true chaperone proteins. Here, we demonstrate that heat shock protein 90β (HSP90β) acts as the most important chaperone in the ocular lens, and plays a critical role in suppressing cataractogenesis. HSP90β is the most abundant chaperone protein in lens and displays drastic change during cataractogenesis. HSP90β silencing in zebrafish causes cataract which cannot be rescued by other HSP90 genes. Mechanistically, HSP90β directly interacts with CHMP4B, a newly found client protein whose upregulation promotes excessive proliferation and also triggers massive apoptosis, leading to microphthalmia and cataractogenesis.

The authors declare no competing interest.

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In vertebrates, there are two major cytoplasmic HSP90: HSP90α and HSP90β. While HSP90α is an inducible protein, HSP90β is a constitutive component of the cytoplasmic proteins (25). Ablation of HSP90α results in viable mice with normal phenotype except for male sterility (26). Knockout of HSP90β, however, leads to embryonic lethality (27), suggesting that HSP90β may be a more important chaperone. Under stress conditions, the cellular HSP90 levels become insufficient to buffer the effects of proteotoxic stresses. It is thus induced to release its client protein, the heat shock transcription factor 1 (HSF1), which homotrimerizes to acquire transcriptional competence (27–33) and promotes excessive HSP90α expression (34, 35). In contrast to HSP90α induction by HSF1, how the constitutive HSP90β expression is transcriptionally regulated remains largely unknown.

In the present study, we demonstrated that HSP90β is the most dominant HSP in the ocular lens. The high level of HSP90β expression is derived from its transcriptional activation by Sp1 family members. More importantly, we show that HSP90β is significantly down-regulated in cataract patients and also in aging mouse lenses. HSP90β ablation in zebrafish results in cataractogenesis which cannot be rescued by three other subtypes of HSP90: cytoplasmic HSP90α, mitochondrial TRAP-1 or Golgi body-contained GRP94. In contrast, silence of HSP90α, TRAP-1, or GRP94 did not cause cataract. HSP90β downregulation due to Sp1 or Sp4 knockdown in zebrafish also lead to microphthalmia and cataractogenesis. Mechanistically, HSP90β directly interacts with CHMP4B, a newly-found client protein whose mutations cause cataract. HSP90β silencing causes upregulation of both CHMP4B, and another client protein, the tumor suppressor p53. Upregulation or overexpression of CHMP4B induces excessive proliferation of lens epithelial cells, and also triggers massive apoptosis of these cells due to activation of the p53/Bak–Bim pathway, leading to cataractogenesis and microphthalmia. Knockdown of both HSP90β and CHMP4B at the same time can rescue HSP90β silencing–induced microphthalmia and cataract. Together, our results reveal that HSP90β is the most abundant HSP in lens and plays a critical role in preventing cataractogenesis through regulation of CHPM4B function and p53-Bak/Bim pathway activation.

Results

HSP90β Is the Most Abundant Chaperone Protein in Human and Zebrafish Lenses. Since HSP90 exists in different isoforms: cytoplasmic HSP90α and HSP90β, mitochondrial TRAP-1 (75 kd) and Golgi body GRP94 (94 kd), we first sought to determine which subtype of HSP90 is the most abundant HSP. To do so, we examined the above 4 subtypes of HSP90 in human lens from eye bank and also from cataract patients. As shown in *SI Appendix*, Fig. S1, HSP90β is the most abundant chaperone, its expression level is about 10-fold to 100-fold higher than the other three subtypes of HSP90. Since zebrafish was used to conduct gene knockdown studies as shown in the later results, we also determined if HSP90β is the most abundant chaperone in zebrafish lens. As shown in *SI Appendix*, Fig. S2, analysis of the mRNA levels for the four subtypes of HSP90 revealed that HSP90β is also the most abundant HSP in zebrafish lens.

HSP90β is Down-Regulated in the Human Cataractous Lens. Knowing that changes in HSP90 expression and activity are associated with various human diseases (21–24), we explore if HSP90 is associated with lens cataractogenesis. To do so, we compared HSP90β in normal and cataractous lens tissues using recently developed WES technology (36–38). Whereas HSP90β is highly expressed in normal transparent lenses, its level was considerably lower in cataractous lenses of the same age groups (Fig. 1 *A* and *B* and *SI Appendix*, Figs. S1 *A* and *E* and S3 *A* and *B*). In contrast, HSP90α, TRAP-1, and GRP94 displayed no such difference between normal and cataractous lenses (*SI Appendix*, Fig. S1 *B–E*).

Among the four heat shock proteins, HSP90β, HSP70, HSP60, and HSP40, HSP90β is also the most abundant HSP in the normal lens (Fig. 1 *A* and *C*), over 10-fold more abundant than HSP70, and over 50-fold more abundant than HSP60 and HSP40 (Fig. 1 *A* and *C*). In human cataractous lenses, HSP90β is over fivefold more abundant than HSP70, and over 50-fold more abundant than HSP60 and HSP40 (*SI Appendix*, Fig. S3). Unlike HSP90β, HSP70, HSP60, and HSP40 showed virtually no differences between normal and cataractous lenses (Fig. 1 *A* and *C*, and *SI Appendix*, Fig. S3). Thus, HSP90β is the most abundant chaperone protein in human ocular lenses, and its downregulation is linked with cataractogenesis.

HSP90β Is Also Down-Regulated in Aging Mouse Lenses. Since cataract is an aging-related disease, we explored if HSP90β would display aging-dependent changes by examining its expression in mouse lenses of different age groups. As shown in Fig. 1 *D–F*, HSP90β is highly expressed in 1-mo-old mouse lenses and is significantly down-regulated in lenses of 8- and 14-mo-old mice.

We also observed HSP90β to be the most abundant HSP in the mouse lens, over 50-fold, 30-fold, and 100-fold more abundant than HSP70, HSP60, and HSP40, respectively. Compared to HSP90β whose expression was decreased by 45%, HSP70, HSP60, and HSP40 showed no age-dependent changes (Fig. 1 *D–F* and *SI Appendix*, Fig. S4).

Sp1 and Sp4 Positively Regulate HSP90β and Are Significantly Down-Regulated in Cataract Patients. To understand why HSP90β is such more abundant than most other HSPs in the ocular lens, we examined its transcriptional regulation. Using the website http://bioinfo.life.hust.edu.cn/hTFtarget/#!/prediction, we searched for transcription factors that may bind to the HSP90β gene (Hsp90ab1) core and proximate promoters (+10 to −804) (Fig. 2*A* and *SI Appendix*, Fig. S5). The highest scored cis-elements were those recognized by Sp1 family members (*SI Appendix*, Fig. S5). In the core and proximate promoter, five putative (M1 to M5) Sp1 family-binding sites were identified (Fig. 2*A*). Sequential deletion and reporter gene assays revealed that four out of the five regions (M1, M2, M4, and M5) likely control HSP90β gene expression (Fig. 2 *A* and *B*). We have carefully examined each of the five HSP90β control regions. As shown in Fig. 2*C* as well as S6 to S10, Sp1 and Sp4 positively regulate HSP90β in lens. Our results identified the major cis-elements for the Sp family transcription factors in the core and proximal promoter of mouse HSP90β gene (Fig. 2*D*). To confirm the control of HSP90β expression by Sp1 family transcription factors, we knocked down Sp1/Sp3/Sp4 in mouse lens epithelial cells, and observed the reduced expression of HSP90β in Sp1/4 knockdown cells, and an opposite result in Sp3 knockdown cells (Fig. 2 *E* and *J*).

Since HSP90β is significantly down-regulated in cataract patients, we speculate that both Sp1 and SP4 may be also down-regulated in these patients. To confirm this hypothesis, we compared the Sp1 and Sp4 levels in normal human lens and cataract patients. As shown in Fig. 2 *K* through 2 *N*, Sp1 and Sp4 expressions were indeed significantly down-regulated in human cataractous lens compared with normal transparent lenses of the same age group. Together, our results demonstrated that high level of HSP90β expression in lens seems to be largely derived from its positive regulation by Sp1 and Sp4.

Silence of HSP90β but Not HSP90α, TRAP-1, or GRP94 in Zebrafish Causes Cataractogenesis. The above results suggest that HSP90β may play an important role in preventing lens

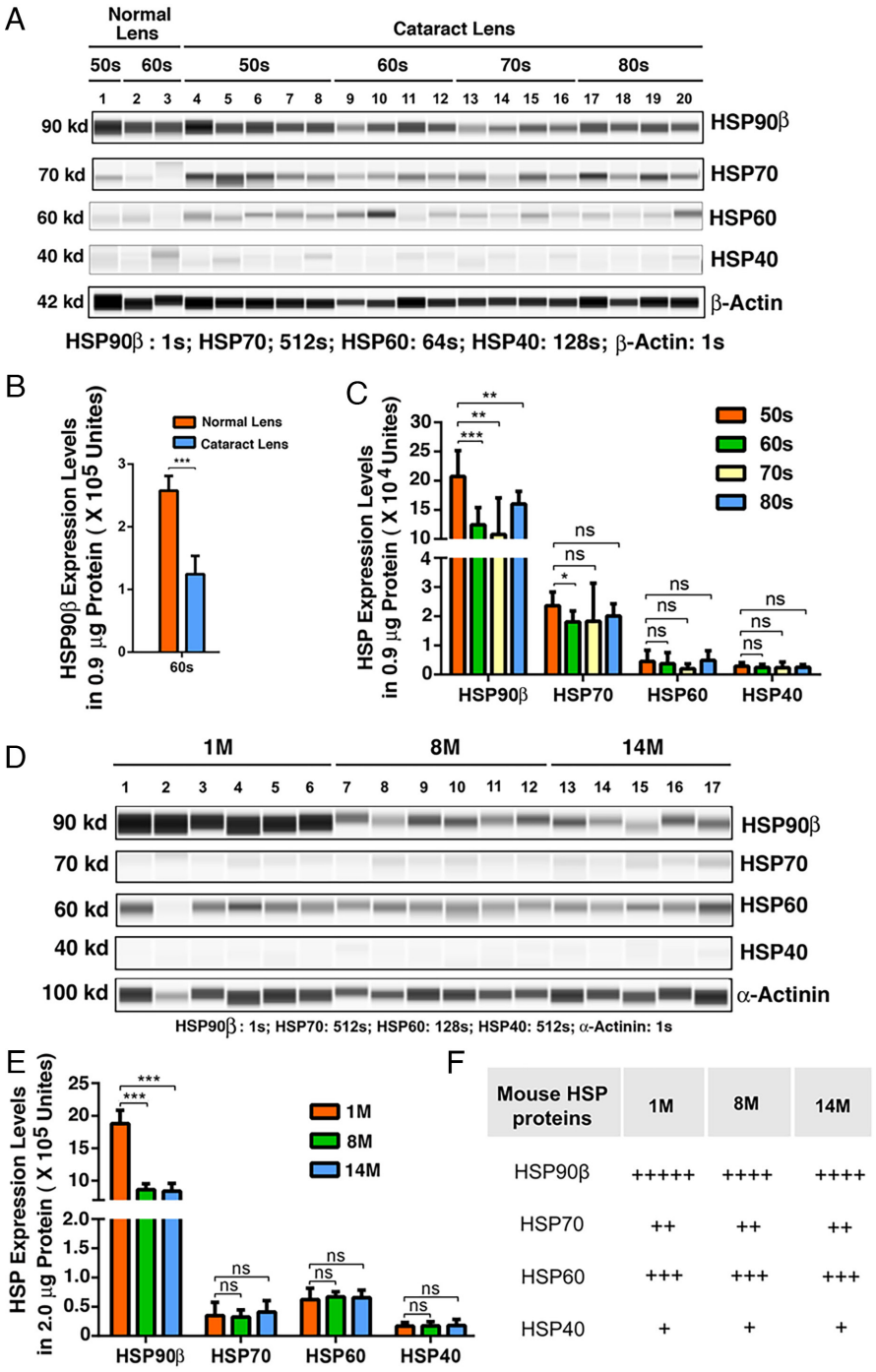

Fig. 1. HSP90β is the most dominant HSP in normal human and mouse lenses and becomes significantly down-regulated during human cataractogenesis and aging of mouse lens. (*A*) The automated western immunoblot (WES) analysis of HSP90β, HSP70 (the HSP70 antibody recognizing both inducible and constitutive isoforms), HSP60 and HSP40 between normal lens and cataract patients in different age groups. Output western blot style data of four HSPs and the β-Actin (as control) with the exposure time indicated. Each lane was loaded 0.9-μg protein. (*B*) Quantification data derived from the software-calculated average of seven exposures (1-512 s). The quantification results show that HSP90β is significantly down-regulated from normal human lens to cataractous lens of the same age group (60s group including 61- to 69-y-old subjects). (*C*) Quantification results show the differential expression levels of four HSPs among different age groups of cataract patients (see *SI Appendix*, Tables S2–S5 for details of different age groups). (*D*) The WES analysis of HSP90β, HSP70 (the HSP70 antibody recognizing both inducible and constitutive isoforms), HSP60 and HSP40 in mouse lenses of 1-, 8-, and 14-mo-old animals. Output western blot style data of four HSPs and the α-Actinin (as control) with the exposure time indicated. Each lane was loaded 2-μg protein. (*E*) Quantification data derived from the software-calculated average of seven exposures (1-512s). The quantification results show that HSP90β is significantly down-regulated from normal mouse lens to aged mouse lens (8- to 14-mo). In contrast, other three HSPs display no age-dependent changes. (*F*) Summary of the WES analysis results of four HSPs in normal and aging mouse lenses. +++++, ++++, +++, ++ and + refer to expression levels of 1×10^6, 1×10^5 to 1×10^6, 5×10^4 to 1×10^5, 3×10^4 to 5×10^4, and less than 3×10^4 units, respectively, in 2-μg total protein. Error bar represents SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, statistically not significant.

pathogenesis. To test this possibility, we silenced HSP90β in zebrafish with morpholino oligos (Fig. 3*A*). As shown in Fig. 3*B*, morpholino oligos targeting exon 3 and 4 of HSP90β significantly reduced its expression. Of note, the HSP90β-silenced zebrafish eye displayed microphthalmia and cataract (Fig. 3*C*). Further characterization revealed a 60% decrease in the lens area/eye area ratio after HSP90β silencing compared with the eye of mock-morpholino oligo-treated zebrafish (Fig. 3 *D* and *E*). In contrast, silence of HSP90α, TRAP-1, or GRP94 in zebrafish did not affect eye phenotypes (*SI Appendix*,

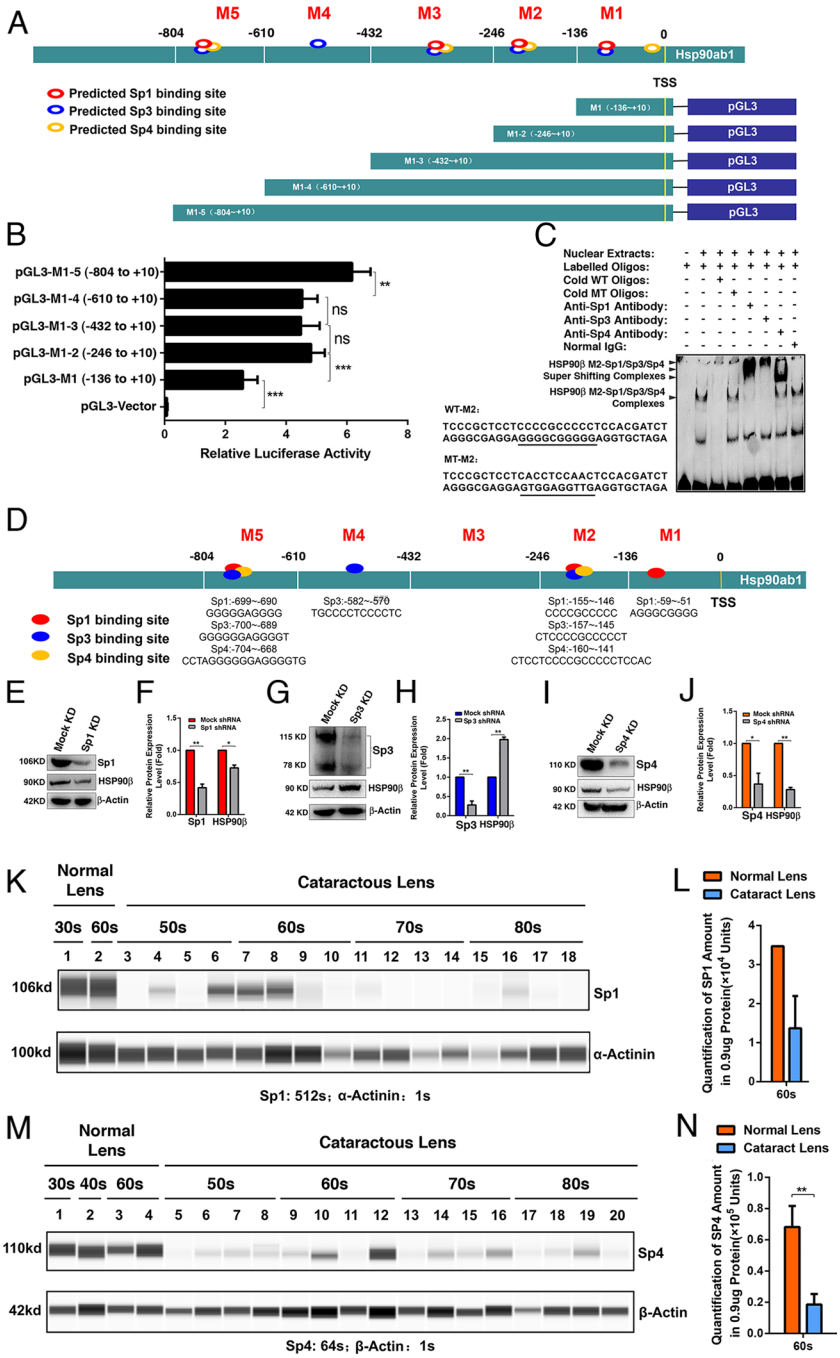

Fig. 2. HSP90β is positively regulated by Sp1 and Sp4. (*A*) A schematic diagram to show: 1) Predicted Sp1/Sp3/Sp4-binding sites in five regions (M1 to M5) of the HSP90β gene core and proximal promoter; 2) the reporter gene constructs covering M1, M1-M2, M1-M3, M1-M4, and M1-M5 regions. (*B*) HSP90β gene core and proximal promoter activities were tested using pGL3 reporter constructs containing different length of promoter fragments as indicated in Fig. 1*A* in αTN4-1 cells. (*C*) Gel mobility shifting assays (EMSA) revealed that Sp1/3/4 can directly bind to the M2 region. The probe sequences are listed in the left, and the two oligos contain either a well-conserved wild-type Sp1/3/4-binding site (WT-M2, *Top*) or mutated Sp1/3/4-binding sites (MT-M2, *Bottom*); The super-shifting bands formed by adding anti-Sp1/3/4 antibodies help to determine the overlapping cis-elements for Sp1/3/4 binding. (*D*) Map of Sp1/3/4-binding sites in HSP90β core and proximal promoter. (*E*) Western blot analysis showed silence of Sp1 by specific shRNA in mouse lens epithelial cells, αTN4-1 downregulates the expression level of HSP90β. (*F*) Quantitative results of Western blots (*E*). (*G*) Western blot analysis showed silence of Sp3 by specific shRNA in mouse lens epithelial cells, αTN4-1 upregulates the expression level of HSP90beta. (*H*) Quantitative results of Western blots (*G*). (*I*) Western blot analysis showed silence of Sp4 by specific shRNA in mouse lens epithelial cells, αTN4-1 also downregulates the expression level of HSP90beta. (*J*) Quantitative results of Western blots (*I*). (*K*–*N*) WES analysis of Sp1(*K* and *L*) and Sp4(*M* and *N*) between normal lens and cataract patients in different age groups. (*K*) Output western blot style data of Sp1 with exposure time indicated. (*L*) Quantitative results of Sp1 derived from the software-calculated average of seven exposures (1-512 s) to show the difference of Sp1 expression in human normal and cataract lenses of the 60s age group. (*M*) Output western blot style data of Sp4 with exposure time indicated. (*N*) Quantitative results of Sp4 derived from the software-calculated average of seven exposures (1-512 s) to show the difference of Sp4 expression in human normal and cataract lenses of the 60s age group. Error bar represents SD. $^{*}P < 0.05$; $^{**}P < 0.01$.

Fig. S11). Moreover, silence of HSP90β can only be rescued by itself but not by HSP90α, TRAP-1, or GRP94 (*SI Appendix*, Fig. S12). Together, we demonstrated that HSP90β is a critical inhibitor of cataractogenesis.

Downregulation of HSP90 Due to Silence of Sp1 or Sp4 in Zebrafish or Due to Aging in the Mouse Causes Cataract or Enhances the Severity of Cataract. Our earlier results revealed that Sp1 and Sp4 play important roles in regulating expression of HSP90β (Fig. 2

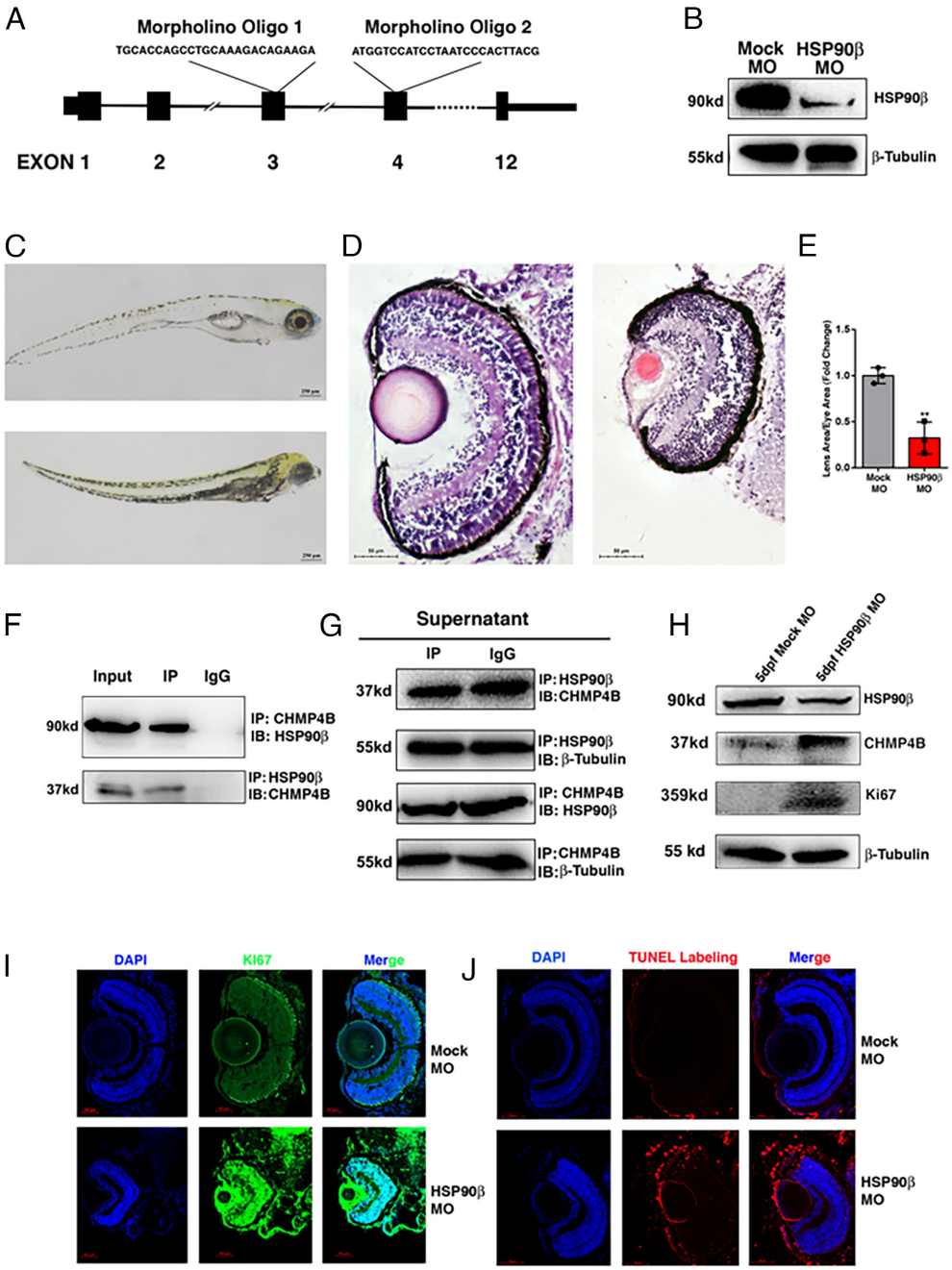

Fig. 3. Silence of HSP90βcauses cataractogenesis in zebrafish lens. (*A*) Two morpholino oligos targeting exon 3 and exon 4 in Zebrafish were designed to silence HSP90β expression. (*B*) Western blot analysis of HSP90β levels in mock-MO and HSP90β-MO treated 5-d postfertilization (dpf) zebrafish embryo. (*C*) Morphology of 5 dpf mock-MO (*Top*) and HSP90β-MO (*Bottom*) zebrafish larvae. (*D*) H.E. staining of 5dpf mock-MO (*Left*) and HSP90β-MO (*Right*) zebrafish eye by frozen sectioning. (*E*) Quantification of the ratio between lens and eye size in mock-MO and HSP90β-MO 5dpf zebrafish embryos. (*F*) Co-IP between HSP90β and CHMP4B, endogenous HSP90β and CHMP4B were precipitated by CHMP4B and HSP90β antibodies, respectively, and detected by western blot analysis. Input protein [0.05% (*Top*) and 5% (*Bottom*)] were included as control. (*G*) For comparison, supernatant samples of IP were also analyzed by western blot. (*H*) Western blot analysis showed that the expression levels of CHMP4B and Ki67 were clearly up-regulated when HSP90β was silenced by morpholino oligos in zebrafish. (*I*) IF staining of Ki67 showed that cell proliferation is up-regulated in 5dpf HSP90β-MO zebrafish eye than in mock-MO zebrafish eye. (*J*) TUNEL labeling assay was used to detect cell apoptosis in zebrafish eye. Compared to 5dpf mock-MO zebrafish, 5dpf HSP90β-MO zebrafish showed significantly more cell apoptosis in lens epithelium. Error bar represents SD. $^{**}P < 0.01$.

and *SI Appendix*, Figs. S6–S10). To further confirm their critical roles in positively regulating HSP90β, we silenced expression of Sp1 (*SI Appendix*, Fig. S13) and Sp4 (*SI Appendix*, Fig. S14) in zebrafish. It was found that silence of either Sp1 or Sp4 leads to distinct downregulation of HSP90β. As a result, both Sp1- and Sp4-silenced zebrafish developed microphthalmia and cataract (*SI Appendix*, Fig. S13 and Fig. S14).

To further demonstrate that aging-induced downregulation of HSP90β is implicated in cataract development, we have created a genetic cataract model in which the Lys-91 sumoylation site was mutated into arginine (*SI Appendix*, Fig. S15*A*). K91R-p32 Pax6 mice develop spotted cataract in the 2-mo age (*Left panel* of *SI Appendix*, Fig. S15*B*). At the age of 12-mo, these animals developed much severe cataract (*Right panel* of *SI Appendix*, Fig. S15*B*). Western blot analysis revealed that 2-mo K91R-p32 Pax6 mice had a much higher level of HSP90β, and by 12-mo, HSP90β levels in these littermate mice were significantly dropped down (*SI Appendix*, Fig. S15*C*). Thus, we observed that the severity of

cataract is highly associated with distinctly down-regulated expression of HSP90β. Together, our results demonstrate that HSP90β downregulation either from Sp1/Sp4 knockdown or from aging induces development of cataract or enhance the severity of cataract, supporting that HSP90β plays a critical role in preventing cataractogenesis, and both Sp1 and Sp4 are crucial for the distinctly high level of HSP90β expression in lens.

HSP90β Regulation of CHMP4B Level Is Critical in Suppressing Cataractogenesis. Next, we explored the possible mechanism by which HSP90β suppresses cataractogenesis. Mass spectroscopy analysis revealed that HSP90β interacted with CHMP4B, a newly-found client protein (*SI Appendix*, Fig. S16). A recent study has shown that abnormal cytokinesis of lens epithelial cells regulated by CHMP4B-mediated ESCRT complex leads to cataract (39). Reciprocal coimmunoprecipitation (Co-IP) confirmed the interaction between HSP90β and CHMP4B (Fig. 3 *F* and *G*). Remarkably, HSP90β silencing caused upregulation of CHMP4B, as well as the M phase marker Ki67 (Fig. 3*H* and *SI Appendix*, Figs. S13, S14, and S17). The latter probably reflects CHMP4B-stimulated lens epithelial cell division. Immunocytochemistry analysis of the HSP90β-silenced zebrafish lens confirmed that the Ki67 signal was dramatically enhanced compared with mock silenced Zebrafish lens (Fig. 3*I*). Theoretically, an increase in the mitosis index should increase the lens size. However, this was not the case, and the HSP90β-silenced zebrafish displayed severe microphthalmia (Fig. 3 *C* and *D*). To understand this paradox, we performed a TUNEL assay and observed a marked increase in apoptosis in the lens of HSP90β-silenced zebrafish but not in the mock control lens (Fig. 3*J*). These cells displayed retarded differentiation (*SI Appendix*, Fig. S18). We also observed high level of apoptosis in Sp1- and Sp4-silenced zebrafish lenses due to distinct downregulation of HSP90β (*SI Appendix*, Figs. S13 and S14). To further confirm that CHMP4B upregulation play a fundamental role in cataractogenesis, we overexpressed CHMP4B in zebrafish. As shown in *SI Appendix*, Fig. S19, CHMP4B overexpression also caused cataract and microphthalmia. More importantly, in HSP90β and CHMP4B double-knockdown zebrafish, we observed that the phenotype of HSP90β silence was much relieved by the CHMP4B silence. As shown in *SI Appendix*, Fig. S20, the double-knockout zebrafish displayed normal phenotype in the eye, further supporting that CHMP4B plays a critical role in mediating HSP90β suppression of cataractogenesis.

HSP90β Prevents Stress-Induced Apoptosis through Suppression of p53 and Its Downstream Proapoptotic Effectors. To better understand how HSP90β silencing activates apoptosis of lens epithelial cells, we used CRISPR/Cas9 to establish stable cell clone in which one copy of the HSP90β gene was silenced as silencing of both copies of the HSP90β gene in lens cells causes lethality. Heterozygosity of the HSP90β gene (+/–) in αTN4-1 cells was confirmed by DNA sequencing and western blot analysis (*SI Appendix*, Fig. S21). Knockdown of HSP90β significantly increased staurosporin or GO-induced apoptosis (*SI Appendix*, Fig. S21).

RNAseq analysis of HSP90β (+/+) mock knockdown cells and HSP90β (+/–) knockdown cells revealed a total of 1,305 genes with altered expression patterns (*SI Appendix*, Fig. S22*A*). Since HSP90β is a chaperone protein, these extensive transcriptomic alterations are likely to be caused by HSP90β-regulated transcription factors. We investigated the possible involvement of p53, a known HSP90β client protein (40). Among the 1,305 genes, we examined 18 p53 target genes (Fig. 4*A* and *SI Appendix*, Fig. S22*B*). QRT-PCR confirmed that 3 out 18 genes: Trp53 (coding for p53), Bak1(coding for Bak), and Bcl2L11 (coding for Bim) displayed significant changes at the mRNA expression. As shown in Fig. 4*B*, HSP90β knockdown up-regulated the mRNAs for p53, Bak, and Bim. Western blot analysis confirmed that knockdown of HSP90β (+/–) significantly up-regulated p53, Bak, and Bim proteins (Fig. 4 *C* and *D*). In contrast, HSP90β knockdown did not change Mcl-1 and Bax expression (*SI Appendix*, Fig. S23)

To determine if altered p53, Bak, and Bim expression contributed to the differences in apoptosis susceptibility between HSP90β (+/+) and HSP90β (+/–) cells, we generated double knockouts: HSP90β (+/–)/p53 (+/–), HSP90β (+/–)/Bak (–/–) and HSP90β (+/–)/Bim (–/–) (*SI Appendix*, Figs. S24–S26). As expected p53, Bak, and Bim knockouts reduced the apoptosis susceptibility of cells with reduced HSP90β expression (Fig. 4 *E* and *M*).

Together, our results demonstrate that HSP90β prevents stress-induced apoptosis of lens epithelial cells via suppression of p53-Bak/Bim pathway activation.

Discussion

The present study shows that HSP90β is the most abundant HSP in lens and that it is critical for the prevention of cataractogenesis. The lens protective activity of HSP90β is mediated by suppression of CHMP4B and p53 (Fig. 4*N*).

HSP90β Is a Critical Inhibitor Preventing Cataractogenesis. Cataract is a complicated ocular disease arising from multiple factors (1, 8). Aging and stress factors are the main reasons for age-related cataract in addition to genetic mutations (8). Structurally, high concentrations of crystallin proteins consisting of α-, β-, and γ-crystallins in lens fiber cells arranged in short order contribute to lens transparency and refractive properties (41, 42). During aging process, protein aggregation is the single most important factor in cataract formation. (43, 44). Factors that lead to protein aggregation include cumulative damage of environmental effects acting in concert with the genetic predisposition encoded in genes for lens proteins (45–47). In addition, lens crystallins show multiple types of modifications with aging of the lens, most of which are caused or accelerated by oxidative free radicals, UV, osmotic, or other types of stresses (8, 48–52). These modifications include proteolysis, an increase in disulfide bridges, phosphorylation, nonenzymatic glycosylation, carbamylation, deamidation of asparagine and glutamine residues, and racemization of aspartic acid residues among others (48–52), leading to loss of the normally stable protein fold and form irreversible aggregates. The slowly denaturing β- and γ-crystallins are bound by α-crystallins, and formation of the complex of the denatured βγ-crystallins by α-crystallins helps to maintain their solubility and thus reduces light scattering. However, α-crystallins lack the ATPase to correct the misfolded βγ-crystallins and thus are not capable to recycle them into the cytoplasm as true chaperones do. As a result, the denatured crystallins are held in complexes with α-crystallins that increase in size to form high molecular weight aggregates, leading to scatter light (53, 54). Overtime, as the high level of α-crystallin in the lens is exhausted, the high molecular weight aggregates precipitate, leading to cataractogenesis. On the other hand, a strong chaperone function would help to resolve this issue to keep lens transparency. In the present studies, we demonstrate that HSP90β has such function to prevent cataractogenesis. Our results show that HSP90β is the most abundant chaperone in lens. It is the only HSP protein that displays distinct difference between normal transparent lens and human cataract lens, and also between young and aged lenses in mouse. Moreover, HSP90β silencing causes microphthalmia and

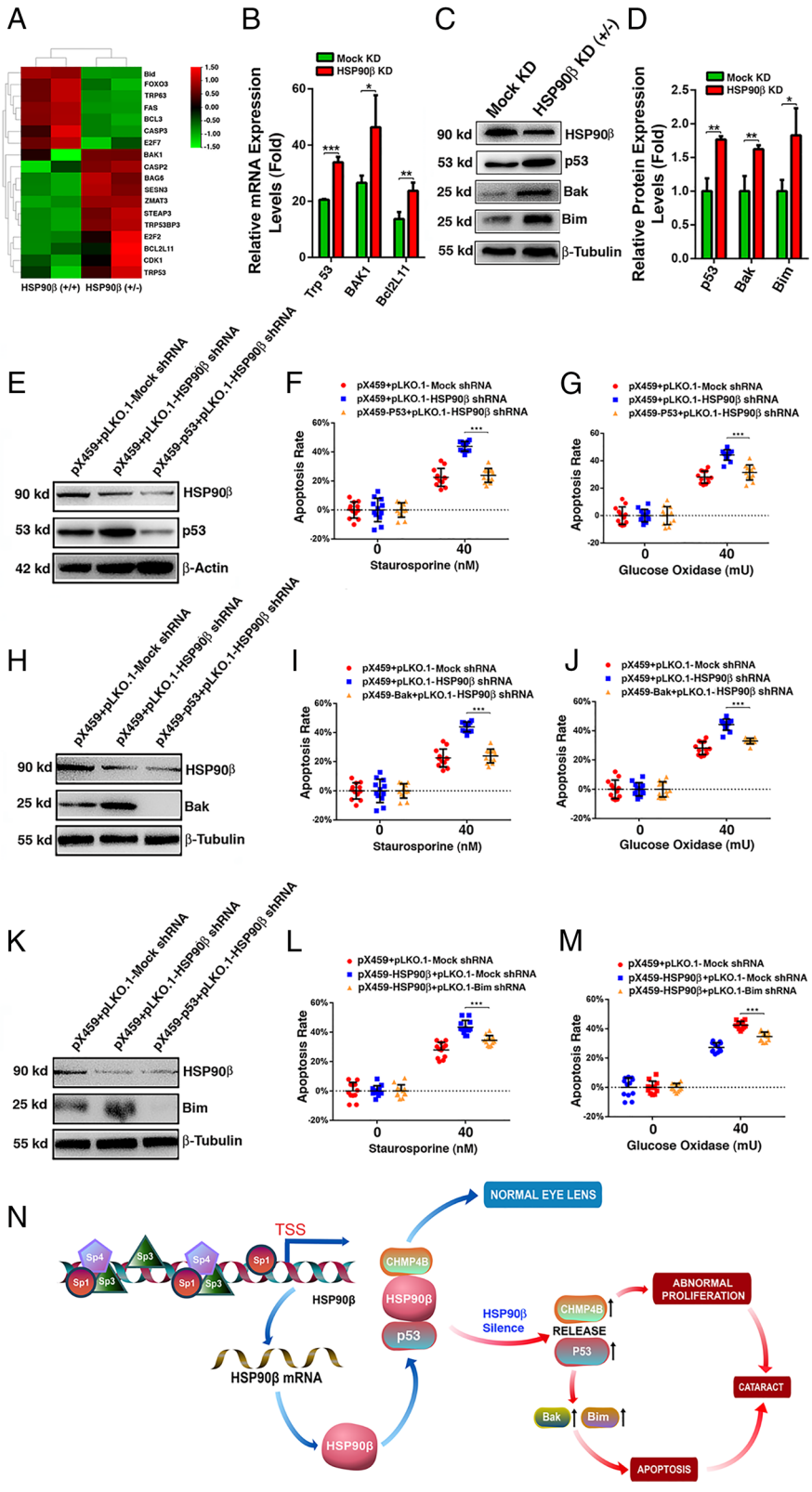

Fig. 4. HSP90β prevents stress-induced apoptosis through suppression of the p53-Bak/Bim pathway activation. (*A*) Hierarchical cluster analysis of apoptosis-associated genes from RNAseq analysis of pX459-HSP90β-αTN4-1 (HSP90β+/−) and pX459-αTN4-1 (HSP90β+/+) cells. (*B*) qRT-PCR analysis verified the mRNA expression levels of p53, Bak, and Bim in HSP90β (+/+) αTN4-1 cells and HSP90β (+/−) αTN4-1 cells. (*C*) Western blot analysis of the expression levels of p53, Bak and Bim in HSP90β (+/+) αTN4-1 cells and HSP90β (+/−) αTN4-1 cells. Note that the expression levels of p53, Bak, and Bim were up-regulated with the downregulation of HSP90β. (*D*) Quantitative results of (*C*). (*E*) Western blot analysis of HSP90β and p53 in pX459+pLKO.1 vectors-αTN4-1 cells [HSP90β (+/+)/p53 (+/+)], pX459+pLKO.1-HSP90β shRNA-αTN4-1 cells [HSP90β (+/−)/p53 (+/+)] and pX459-p53+pLKO.1-HSP90β shRNA-αTN4-1 cells [HSP90β (+/−)/p53 (+/−)]. (*F* and *G*) Differential apoptosis rates in the above three types of cells without or with treatment by 40 nM staurosporine or 40 mU/mL GO. The cell viability is measured by the Cell Titer-Lumi™ Luminescent Cell Viability Assay Kit. (*H*) Western blot analysis of HSP90β and Bak in pX459+pLKO.1 vectors-αTN4-1 cells [HSP90β (+/+)/Bak (+/+)], pX459+pLKO.1-HSP90β shRNA-αTN4-1 cells [HSP90β (+/−)/Bak (+/+)] and pX459-Bak+pLKO.1-HSP90β shRNA-αTN4-1 cells [HSP90β (+/−)/Bak (−/−)]. (*I* and *J*) Differential apoptosis rates in the above three types of cells without or with treatment by 40 nM staurosporine or 40 mU/mL GO. The cell viability is measured by the Cell Titer-Lumi™ Luminescent Cell Viability Assay Kit. (*K*) Western blot analysis of HSP90β and Bim in pX459+pLKO.1 vectors-αTN4-1 cells [HSP90β (+/+)/Bim (+/+)], pX459+pLKO.1-HSP90β shRNA-αTN4-1 cells [HSP90β (+/−)/Bim (+/+)] and pX459-Bim+pLKO.1-HSP90β shRNA-αTN4-1 cells [HSP90β (+/−)/Bim (−/−)]. (*L* and *M*) Differential apoptosis rates in the above three types of cells without or with treatment by 40 nM staurosporine or 40 mU/mL GO. The cell viability is measured by the Cell Titer-Lumi™ Luminescent Cell Viability Assay Kit. Note that knockout of p53, Bak, or Bim attenuates staurosporine- or GO-induced apoptosis in HSP90β (+/−)-αTN4-1 cells, suggesting that HSP90β prevents stress-induced apoptosis through suppression of p53-Bak/Bim pathway activation. (*N*) Summary of HSP90β regulation and functions in the ocular lens. Under nonstress induction conditions, the dominant expression of HSP90β is mainly regulated by the Sp1 family transcription factors. The high level of HSP90β can interact with CHMP4B, a newly-found client protein, and p53 to maintain normal cell proliferation and survival to keep lens transparency. Silence of HSP90β induces upregulation of CHMP4B and p53. The increased expression of CHMP4B promotes cell proliferation, and accumulation of the excess number of lens epithelial cells without proper differentiation will cause cataractogenesis. On the other hand, p53 upregulation enhances expression of Bim and Bak, triggering apoptosis of lens epithelial cells, leading to cataractogenesis. Error bar represents SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

cataractogenesis but not other subtypes of HSP90. Furthermore, HSP90β downregulation derived from knockdown of Sp1 or Sp4 also leads to microphthalmia and cataractogenesis. Finally, HSP90β deficiency–induced cataract and microphthalmia can only be rescued by itself but not by other subtypes of HSP90. Thus, we demonstrate that HSP90β is a critical inhibitor of cataractogenesis in ocular lens. Lack of reports that mutations of the HSP90β gene cause cataracts is likely due to the fact that such mutants may be embryonic lethal since deletion of HSP90β in mice leads to embryonic lethality (27). A strong HSP90β chaperone could guard the lens from damages by stress effects, and moreover, is capable of correcting the misfolded proteins and recycling them into cytoplasm by consuming ATP. In addition to its strong chaperone activity to prevent protein aggregation,

HSP90β can also suppresses stress-induced apoptosis of lens epithelial cells through its control on p53 and its downstream targets (Fig. 3). As a result, it can preserve the functions of lens epithelial cells to maintain lens transparency. Taking together, we have shown that HSP90β is a critical inhibitor preventing cataractogenesis in lens.

Sp1 and Sp4 Are Important Transcription Factors Regulating both Lens Differentiation and Pathogenesis. The Sp1 family of transcription factors interact with GC/GT oligonucleotide sequences existing in diverse viral and cellular gene promoters (55, 56). These members not only have highly conserved DNA-binding domains but also bear high levels of sequence homology in other regions (57). Previous studies have shown that the Sp1 transcription factors regulate many targets (58). We and others have shown that during lens development, Sp1 regulates expression of δ- and β-crystallin genes to mediate lens differentiation (59, 60). The function of Sp1 is finely regulated by differential modulations by SUMO1 and SUMO2/3 (60).

In the present study, we demonstrated that among different HSPs (HSP90β, HSP70, HSP60, and HSP40), HSP90β is the most abundant HSP in human and mouse lens epithelial cells. This dominantly high level of expression is derived from the positive regulation by Sp1 and Sp4 of the specificity protein 1 (Sp1) family transcription factors through interactions with multiple cis-elements in the proximal and core promoter of HSP90β gene.

Despite its abundant expression in the young and healthy lens, HSP90β is significantly down-regulated during aging and cataractogenesis. This downregulation is derived from diminished Sp1 and Sp4 expression as observed in human cataract lenses. Our demonstration that knockdown of Sp1 or Sp4 in zebrafish distinctly down-regulates HSP90β further support this conclusion. Importantly, HSP90β downregulation is functionally significant because its silencing in zebrafish leads to microphthalmia and cataract formation. Our demonstration that Sp1 or Sp4 silencing also causes microphthalmia and cataract indicates that they are critical transcription factors in lens and play fundamental roles in both lens development and transparency.

HSP90β Regulation of CHMP4B Level Has a Critical Role in Suppressing Cataractogenesis. The importance of the charged multivesicular body protein 4B (CHMP4B) in lens transparency was initially demonstrated in a congenital cataract patient where in exon 3 of the gene, a nonconservative substitution of a valine residue for aspartic acid residue at codon 129 (p.D129V) occurs (61). Subsequently, it was found that CHMP4B regulates lens growth and differentiation (62). More recently, CHMP4B was found to play an indispensable role in cytokinesis of lens epithelial cell proliferation (39). In the present study, we demonstrated that CHMP4B is a newly-found client protein of HSP90β, and plays an important role in mediating HSP90β suppression of cataractogenesis. HSP90β silencing induces significant upregulation of CHMP4B through a yet to be determined mechanism. Upregulation of CHMP4B is accompanied by increased expression of the cell cycle marker Ki67, but the connection between CHPM4B and Ki67 may be indirect. More importantly, overexpression of CHMP4B alone causes microphthalmia and cataractogenesis, similar phenotypes as HSP90β silencing displayed. Furthermore, we observed that double silence of HSP90β and CHMP4B simultaneously restored the normal eye phenotypes. These experimental results strongly support that HSP90β regulation of CHMP4B level plays a critical role in suppressing cataractogenesis. Either upregulation derived from HSP90β silencing or overexpression of CHMP4B alone promotes aberrant lens cell division, which is accompanied by apoptosis rather than an increase in lens size. We also observed that the proliferated lens cells derived from CHMP4B upregulation displayed clear retarded differentiation. The apoptosis appears to be mediated by p53, previously identified as another HSP90 client protein (40). Whether the upregulation of p53 is a direct consequence of HSP90 downregulation or a compensatory response to aberrant mitosis remains to be determined. Nonetheless, p53 knockdown protects HSP90 deficient lens cells from excessive apoptosis.

In summary, our results presented in this study demonstrate that HSP90β is a critical inhibitor of cataract, a leading ocular disease that causes global blindness. It prevents cataract formation through negative regulation of CHMP4B, a newly-identified client protein, and p53/Bim-Bak pathway activation.

Methods

Animals. Mice and zebrafish used in this study were handled in compliance with the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington, DC). Two-month and 12-mo-old p32 Pax6-K91R mice and zebrafish of different ages were raised in the animal facility of Sun Yat-sen University.

Collection of Human Lens Capsular Epithelia. The normal control lenses were dissected from donor eye balls supplied by Guangdong Province Eye Bank, and the lens epithelia were removed from these eye balls and used for RNA and protein extraction. Ethical approval to use human tissues was granted by the Ethics Committee of Zhongshan Ophthalmic Center. Collection of cataract lens capsular epithelia from different age groups of patients was conducted with patients' consent and also approved by the above Committee as previously described (37, 38). The detail information of the epithelial samples was described in *SI Appendix*, Tables S1–S5.

Statistical Analysis. In the present study, all the data presented are derived from at least three experiments. During data analysis, statistical analysis was conducted for all sets of data when necessary. Both averages and SDs were calculated, and statistical analysis was conducted as previously described (63–65).

Ethics Approval Statement. All authors approve this submission.

Patient Consent Statement. Patient consent has been obtained from all patients.

Data, Materials, and Software Availability. All data are included in the manuscript and/or *SI Appendix*.

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1. D. G. Cogan, The lens, cataracts, and galactosemia. *N. Engl. J. Med.* **288**, 1239–1240 (1973).
2. P. Periyasamy, T. Shinohara, Age-related cataracts: Role of unfolded protein response, Ca(2+) mobilization, epigenetic DNA modifications, and loss of Nrf2/Keap1 dependent cytoprotection. *Prog. Retin Eye Res.* **60**, 1–19 (2017).
3. K. L. Schey, Z. Wang, M. G. Friedrich, D. L. Garland, R. J. W. Truscott, Spatiotemporal changes in the human lens proteome: Critical insights into long-lived proteins. *Prog. Retin Eye Res.* **76**, 100802 (2020).
4. P. J. Donaldson, Y. Chen, R. S. Petrova, A. C. Grey, J. C. Lim, Regulation of lens water content: Effects on the physiological optics of the lens. *Prog. Retin Eye Res.* 101152 (2022), 10.1016/j.preteyeres.2022.101152, 101152.
5. J. Liu *et al.*, Mechanosensitive collaboration between integrins and connexins allows nutrient and antioxidant transport into the lens. *J. Cell Biol.* **219**, e202002154 (2020).
6. Y. Quan, Y. Du, C. Wu, S. Gu, J. X. Jiang, Connexin hemichannels regulate redox potential via metabolite exchange and protect lens against cellular oxidative damage. *Redox Biol.* **46**, 102102 (2021).
7. H. R. Taylor *et al.*, Effect of ultraviolet radiation on cataract formation. *N. Engl. J. Med.* **319**, 1429–1433 (1988).
8. A. Shiels, J. F. Hejtmancik, Biology of Inherited Cataracts and Opportunities for Treatment. *Annu. Rev. Vis. Sci.* **5**, 123–149 (2019).
9. W. C. Li *et al.*, Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. *J. Cell Biol.* **130**, 169–181 (1995).
10. W. C. Li, J. R. Kuszak, G. M. Wang, Z. Q. Wu, A. Spector, Calcimycin-induced lens epithelial cell apoptosis contributes to cataract formation. *Exp Eye Res.* **61**, 91–98 (1995).
11. W. C. Li, A. Spector, Lens epithelial cell apoptosis is an early event in the development of UVB-induced cataract. *Free Radic. Biol. Med.* **20**, 301–311 (1996).
12. Q. Yan, J. P. Liu, D. W. Li, Apoptosis in lens development and pathology. *Differentiation* **74**, 195–211 (2006).
13. U. P. Andley, Crystallins in the eye: Function and pathology. *Prog. Retin Eye Res.* **26**, 78–98 (2007).
14. J. Horwitz, Alpha-crystallin can function as a molecular chaperone. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10449–10453 (1992).
15. P. Mehlen, C. Kretz-Remy, X. Préville, A. P. Arrigo, Human hsp27, Drosophila hsp27 and human alphaB-crystallin expression-mediated increase in glutathione is essential for the protective activity of these proteins against TNFalpha-induced cell death. *Embo J.* **15**, 2695–2706 (1996).
16. Y. W. Mao, J. P. Liu, H. Xiang, D. W. Li, Human alphaA- and alphaB-crystallins bind to Bax and Bcl-X(S) to sequester their translocation during staurosporine-induced apoptosis. *Cell Death Differ* **11**, 512–526 (2004).
17. J. P. Liu *et al.*, Human alphaA- and alphaB-crystallins prevent UVA-induced apoptosis through regulation of PKCalpha, RAF/MEK/ERK and AKT signaling pathways. *Exp Eye Res.* **79**, 393–403 (2004).
18. D. W. Li *et al.*, Calcium-activated RAF/MEK/ERK signaling pathway mediates p53-dependent apoptosis and is abrogated by alpha B-crystallin through inhibition of RAS activation. *Mol. Biol. Cell* **16**, 4437–4453 (2005).
19. F. H. Schopf, M. M. Biebl, J. Buchner, The HSP90 chaperone machinery. *Nat. Rev. Mol. Cell Biol.* **18**, 345–360 (2017).
20. M. Taipale, D. F. Jarosz, S. Lindquist, HSP90 at the hub of protein homeostasis: Emerging mechanistic insights. *Nat. Rev. Mol. Cell Biol.* **11**, 515–528 (2010).
21. E. M. Alexandrova *et al.*, Improving survival by exploiting tumour dependence on stabilized mutant p53 for treatment. *Nature* **523**, 352–356 (2015).
22. C. A. Dickey *et al.*, The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. *J. Clin. Invest.* **117**, 648–658 (2007).
23. X. Wang *et al.*, Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. *Cell* **127**, 803–815 (2006).
24. Y. Wu *et al.*, The molecular chaperone Hsp90α deficiency causes retinal degeneration by disrupting Golgi organization and vesicle transportation in photoreceptors. *J. Mol. Cell Biol.* **12**, 216–229 (2020).
25. C. Prodromou, Mechanisms of Hsp90 regulation. *Biochem. J.* **473**, 2439–2452 (2016).
26. I. Grad *et al.*, The molecular chaperone Hsp90α is required for meiotic progression of spermatocytes beyond pachytene in the mouse. *PLoS One* **5**, e15770 (2010).
27. S. E. Jackson, Hsp90: Structure and function. *Top Curr. Chem.* **328**, 155–240 (2013).
28. K. Abravaya, M. P. Myers, S. P. Murphy, R. I. Morimoto, The human heat shock protein hsp70 interacts with HSF, the transcription factor that regulates heat shock gene expression. *Genes Dev.* **6**, 1153–1164 (1992).
29. R. Baler, W. J. Welch, R. Voellmy, Heat shock gene regulation by nascent polypeptides and denatured proteins: hsp70 as a potential autoregulatory factor. *J. Cell Biol.* **117**, 1151–1159 (1992).
30. R. Baler, G. Dahl, R. Voellmy, Activation of human heat shock genes is accompanied by oligomerization, modification, and rapid translocation of heat shock transcription factor HSF1. *Mol. Cell Biol.* **13**, 2486–2496 (1993).
31. R. Baler, J. Zou, R. Voellmy, Evidence for a role of Hsp70 in the regulation of the heat shock response in mammalian cells. *Cell Stress Chaperones* **1**, 33–39 (1996).
32. E. A. Craig, C. A. Gross, Is hsp70 the cellular thermometer? *Trends Biochem. Sci.* **16**, 135–140 (1991).
33. K. D. Sarge, S. P. Murphy, R. I. Morimoto, Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. *Mol. Cell Biol.* **13**, 1392–1407 (1993).
34. J. Zou, Y. Guo, T. Guettouche, D. F. Smith, R. Voellmy, Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* **94**, 471–480 (1998).
35. E. J. Solís *et al.*, Defining the Essential Function of Yeast Hsf1 Reveals a Compact Transcriptional Program for Maintaining Eukaryotic Proteostasis. *Mol. Cell* **63**, 60–71 (2016).
36. J. A. Dahl *et al.*, Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature* **537**, 548–552 (2016).
37. F. Y. Liu *et al.*, Molecular signature for senile and complicated cataracts derived from analysis of sumoylation enzymes and their substrates in human cataract lenses. *Aging Cell* **19**, e13222 (2020).
38. L. Wang *et al.*, PP-1β and PP-2Aα modulate cAMP response element-binding protein (CREB) functions in aging control and stress response through de-regulation of αB-crystallin gene and p300–p53 signaling axis. *Aging Cell* **20**, e13458 (2021).
39. F. Gulluni *et al.*, PI(3,4)P2-mediated cytokinetic abscission prevents early senescence and cataract formation. *Science* **374**, eabk0410 (2021).
40. C. Wang, J. Chen, Phosphorylation and hsp90 binding mediate heat shock stabilization of p53. *J. Biol. Chem.* **278**, 2066–2071 (2003).
41. G. B. Benedek, Theory of transparency of the eye. *Appl. Opt.* **10**, 459–473 (1971).
42. M. Delaye, A. Tardieu, Short-range order of crystallin proteins accounts for eye lens transparency. *Nature* **302**, 415–417 (1983).
43. H. Bloemendal *et al.*, Ageing and vision: Structure, stability and function of lens crystallins. *Prog. Biophys. Mol. Biol.* **86**, 407–485 (2004).
44. K. L. Moreau, J. A. King, Protein misfolding and aggregation in cataract disease and prospects for prevention. *Trends Mol. Med.* **18**, 273–282 (2012).
45. J. F. Hejtmancik, M. Kantorow, Molecular genetics of age-related cataract. *Exp. Eye Res.* **79**, 3–9 (2004).
46. M. C. Leske *et al.*, Risk factors for nuclear opalescence in a longitudinal study. LSC Group. Longitudinal Study of Cataract. *Am. J. Epidemiol.* **147**, 36–41 (1998).
47. E. Yonova-Doing *et al.*, Genetic and Dietary Factors Influencing the Progression of Nuclear Cataract. *Ophthalmology* **123**, 1237–1244 (2016).
48. K. K. Sharma, P. Santhoshkumar, Lens aging: Effects of crystallins. *Biochim Biophys Acta* **1790**, 1095–1108 (2009).
49. B. K. Derham, J. J. Harding, Alpha-crystallin as a molecular chaperone. *Prog. Retin Eye Res.* **18**, 463–509 (1999).
50. M. F. Lou, Redox regulation in the lens. *Prog Retin Eye Res* **22**, 657–682 (2003).
51. A. Banh, V. Bantseev, V. Choh, K. L. Moran, J. G. Sivak, The lens of the eye as a focusing device and its response to stress. *Prog. Retin Eye Res.* **25**, 189–206 (2006).
52. J. A. Jedziniak, J. H. Kinoshita, E. M. Yates, G. B. Benedek, The concentration and localization of heavy molecular weight aggregates in aging normal and cataractous human lenses. *Exp. Eye Res.* **20**, 367–369 (1975).
53. M. B. Datiles IIIrd. *et al.*, Clinical detection of precataractous lens protein changes using dynamic light scattering. *Arch Ophthalmol.* **126**, 1687–1693 (2008).
54. P. V. Rao, Q. L. Huang, J. Horwitz, J. S. Zigler Jr., Evidence that alpha-crystallin prevents non-specific protein aggregation in the intact eye lens. *Biochim. Biophys. Acta* **1245**, 439–447 (1995).
55. W. S. Dynan, R. Tjian, The promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. *Cell* **35**, 79–87 (1983).
56. D. Gidoni, W. S. Dynan, R. Tjian, Multiple specific contacts between a mammalian transcription factor and its cognate promoters. *Nature* **312**, 409–413 (1984).
57. L. Lania, B. Majello, P. De Luca, Transcriptional regulation by the Sp family proteins. *Int. J. Biochem. Cell Biol.* **29**, 1313–1323 (1997).
58. S. Safe, K. Kim, Nuclear receptor-mediated transactivation through interaction with Sp proteins. *Prog. Nucleic Acid Res. Mol. Biol.* **77**, 1–36 (2004).
59. G. C. Das, J. Piatigorsky, The chicken delta 1-crystallin gene promoter: Binding of transcription factor(s) to the upstream G+C-rich region is necessary for promoter function in vitro *Proc. Natl. Acad. Sci. U.S.A.* **83**, 3131–3135 (1986).
60. L. Gong *et al.*, Sumoylation differentially regulates Sp1 to control cell differentiation. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 5574–5579 (2014).
61. A. Shiels *et al.*, CHMP4B, a novel gene for autosomal dominant cataracts linked to chromosome 20q. *Am. J. Hum. Genet* **81**, 596–606 (2007).
62. Y. Zhou, T. M. Bennett, A. Shiels, A charged multivesicular body protein (CHMP4B) is required for lens growth and differentiation. *Differentiation* **109**, 16–27 (2019).
63. L. Zhang *et al.*, MYPT1/PP1-mediated EZH2 dephosphorylation at S21 promotes epithelial-mesenchymal transition in fibrosis through control of multiple families of genes. *Adv. Sci. (Weinh)* **9**, e2105539 (2022).
64. Q. Yan *et al.*, Sumoylation activates the transcriptional activity of Pax-6, an important transcription factor for eye and brain development. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 21034–21039 (2010).
65. L. Xiao *et al.*, Protein phosphatase-1 regulates Akt1 signal transduction pathway to control gene expression, cell survival and differentiation. *Cell Death Differ* **17**, 1448–1462 (2010).