Identification of Vimentin as a Novel Target of HSF4 in Lens Development and Cataract by Proteomic Analysis

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PURPOSE. To explore the target genes of HSF4, especially those involved in lens developmental processes and cataract formation.

METHODS. A slit lamp biomicroscopy examination was performed on Hsf4flox/flox B-crystallin+/− knockout mice and wild-type mice. Two-dimensional electrophoresis combined with mass spectrometry was used to identify differentially expressed lens proteins between wild-type and Hsf4flox/flox B-crystallin+/− knockout mice and further confirmed by Western blot and immunohistochemistry. Histologic analysis was used to analyze the denucleation process of lens fiber cells. Moreover, an electrophoretic mobility shift assay (EMSA), luciferase assay, and ChIP assay were used to validate the effects of HSF4 on vimentin expression.

RESULTS. Hsf4flox/flox B-crystallin+/− knockout mice had abnormal lenses and developed cataract. The downregulated proteins were major structural proteins including α- and β-crystallins, whereas the upregulated proteins were mainly enzymes and an intermediate filament protein, vimentin. The upregulated vimentin expression level was further confirmed by Western blot, Q-PCR, and immunofluorescence. EMSA, luciferase assay, and ChIP assay validated that HSF4 had DNA-binding ability to vimentin promoter and repressed vimentin expression.

CONCLUSIONS. These findings indicate that HSF4 represses vimentin gene expression via the HSE-like element. The loss of HSF4 function results in an increase in vimentin expression in Hsf4flox/flox B-crystallin+/− knockout mice and affects lens differentiation, particularly impairing the denucleation of lens fiber cells. These events appear to implicate a molecular mechanism in abnormal lens development and cataract formation in Hsf4flox/flox B-crystallin+/− knockout mice. The HSF4-vimentin axis appears to be a new target for developing anti-cataract drugs, especially for those cataracts resulting from aberrations in HSF4 expression. (Invest Ophthalmol Vis Sci. 2010;51:396 – 404) DOI:10.1167/iovs.09-3772

Cells respond to stress signals by rapidly inducing the expression of heat shock proteins (HSPs). The inducible HSPs expression is regulated by heat shock transcriptional factors (HSFs). In response to various stresses, most HSFs acquire DNA binding activity to the heat shock element (HSE) in the promoter of HSPs, thereby mediating transcription of the heat shock genes and resulting in the accumulation of HSPs.1–4 HSE is composed of three contiguous inverted repeats of a 5-bp sequence with a consensus defined as nGAAn.5,6 In mammals, three heat shock transcription factors are expressed: HSF1, -2, and -4.2 All the HSFs have a helix-turn-helix DNA-binding domain and a hydrophobic repeat (HR-A/B) that is essential for trimer formation. HSF trimers bind to HSE with high affinity. In both HSF1 and -2, there is another hydrophobic repeat (HR-C) that suppresses trimer formation. Therefore, HSFs stay as monomers or dimers under normal growth conditions and are converted to trimers when they are activated.6 In contrast to HSF1 and -2 proteins, which are expressed in most tissues, the expression level of HSF4 protein is too low to be detected in many tissues,7 except in lens, in which HSF4 protein expression is extremely high.8 No stress-related functions have been shown for HSF4, but its importance in lens formation and maintenance of olfactory epithelium has been well documented.9,10 HSF4 has six transcripts—HSF4a, -b, -c, -d, -g, and -b—and three apparently aberrantly spliced mRNAs.10 All HSF4 isoforms lack the HR-C domain that inhibits trimer formation. Therefore, HSF4 is constitutively a trimer that binds to HSE. Previous studies showed that β-crystallin is regulated directly by HSF4 and other crystallin genes possess HSF4 binding sites,8,11 suggesting that HSF4 may have physiological roles during development.12 Mutations in HSF4 have been identified in human families with autosomal dominant13,14 or recessive congenital cataracts.15,16 Meanwhile, Hsf4-null mice exhibit defects in lens fiber cell differentiation and early cataract formation.15,16 Early transposable element insertion in intron 9 of the HSF4 gene can also result in autosomal recessive cataracts in lop11 and Ids1 mice.11 Because of differences in Hsf4-null mice construction, differences in the genetic background of the mice have been tested, Fujimoto et al.18 detected markedly reduced expression of γ(A-F)-crystallin genes, whereas Min et al.19 did not observe a significant reduction of γ-crystallin genes except for the γF-crystallin gene. Besides, Shi et al.18 discovered markedly reduced expression of β-crystallin gene while Fujimoto et al. and Min et al. did not. These observations suggest that HSF4 plays a critical role in normal lens development. Although the importance of HSF4 in lens development is evident, the molecular events leading to abnormal lens differentiation and cataract formation are still poorly understood.

It is known that development of the lens occurs via differentiation of peripheral epithelial cells into elongated fiber cells, which accumulate in concentric layers, eventually losing their
nuclei and other organelles. A previous study showed that vimentin interlinks the nucleus to the plasma membrane of lens epithelial cells and usually disappears during the lens fiber cell maturation process. Overexpression of chicken vimentin in the mouse lens impairs the process of denucleation. In the present study, we used the Hsf4tm1Xyk knockout mice to explore the target genes of HSF4, especially those involved in lens developmental processes and cataract formation. Vimentin was identified as one of the direct targets of HSF4 and its expression level was upregulated in Hsf4tm1Xyk knockout mice. These observations suggest that excess accumulation of vimentin may be one of the factors that impair the denucleation process during lens fiber cell maturation and cause lens abnormality or cataract.

**Materials and Methods**

**Animals and Cell Culture**

The use of animals in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Hsf4tm1Xyk knockout mice were generated by another laboratory in our institute. SRA01/04 cells were cultured in Dulbecco's modified Eagle's medium with low glucose (Invitrogen-Gibco, Gaithersburg, MD) and supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin in a humidified incubator at 37°C and 5% CO2/95% air. The mice used in the present study were 8 weeks old, except the few that were specially described.

**Slit Lamp Examination**

Mice were examined with a slit lamp ophthalmoscope (YZ5F, slit lamp; 66 Vision Tech. Co., Ltd., Suzhou, China) with a imaging system (VTXF-QB1A Imaging System; Strong Tech. Co., Ltd., Xiamen, China). After the mice were anesthetized with intraperitoneal 0.2% ketamine and 0.5% xylazine (Wuxi Shanhe Group, Jiangsu, China), the eyes of the mice were washed with 0.3% H2O2 for 30 minutes to block endogenous peroxidase. After they were blocked in 10% goat serum, the sections were incubated with polyclonal rabbit anti-vimentin antibody (1:100; sc-19860; Santa Cruz Biotechnology, Santa Cruz, CA), 2 μL per reaction, was added. The DNA–protein complex was separated in 4% nondenaturing polyacrylamide gels by electrophoresis. Then the DNA-protein complexes were transferred onto nylon membranes and detected by chemiluminescence (Pierce).

**Chromatin Immunoprecipitation Assay (ChIP)**

A ChIP analysis was performed as described previously. With anti-HSF4 antibodies. Immunoprecipitated DNA fragments were analyzed by semiquantitative PCR amplification using primers for vimentin. The primers for vimentin were 5'-GGGGGAGGAGGAGGAGGAGGAG-3' (forward) and 5'-GGAGGGGAGGAGGTGAGGAG-3' (reverse). The PCR product was subcloned into pGL3-basic (Promega, Madison, WI) with BamHI/HindIII sites for pGL3-VimHSF4-luciferase plasmids. The sequence of the clones was verified by DNA sequencing.

**Luciferase Assay**

SRA01/04 cells were seeded in a 24-well plate and transfected with indicated amount of pcDNA3.1-HSF4b together with pGL3-VimPro-luciferase (250 ng), luciferase reporter containing an HSE-like sequence of vimentin promoter and pRL-TK Renilla (10 ng). Twenty-four hours later, the cell lysates were analyzed by luciferase assay (Dual-Luciferase Assay system; Promega), according to the manufacturer’s instructions. The relative luciferase activity was normalized by pRL-TK Renilla activity.

**Quantitative Real-Time PCR (Q-PCR)**

Total RNA extraction and Q-PCR reactions were performed as described previously. The primers for vimentin were 5'-ACCCGTTTGGCAGCTAGCAT-3' (forward) and 5'-TGTGCGGTGGGTGCTGG-3' (reverse), with HPR as the internal control. The primers for HPR were 5'-CCAGGCTGTTAGG-3' (forward) and 5'-GCCCTCATGTTG-3' (reverse). The protein extracts of mouse lenses (20 μg protein each) were subjected to 10% SDS–polyacrylamide gel and transferred to nitrocellulose membrane (Bio-Rad). After the membrane was blocked in 5% nonfat milk, it was incubated with goat anti-HSF4 (1:1000; sc-19860; Bio-Rad). Western Blot Analysis

The protein extracts of mouse lenses (20 μg protein each) were subjected to 10% SDS–polyacrylamide gel and transferred to nitrocellulose membrane (Bio-Rad). After the membrane was blocked in 5% nonfat milk, it was incubated with goat anti-HSF4 (1:1000; sc-19860; Bio-Rad).
were confirmed in the Hsf4tm1Xyk mouse eyeball. The anterior chamber of previously reported, smaller lens and cataract formation mice and wild-type mice under slit lamp biomicroscopy. As microscopic side views of a knockout mice was obviously smaller than that of the wild-type mice.

**RESULTS**

All experiments were repeated at least three times. Data are expressed as the mean ± SE. Student’s t-test was used to compare the difference between two groups. P < 0.05 was considered to be statistically significant.

**Defects in Eye Lens Development in Hsf4tm1Xyk-Knockout Mice**

To assess lens development and cataract formation, we examined the anterior segments of the eyes of Hsf4tm1Xyk-knockout mice and wild-type mice under slit lamp biomicroscopy. As previously reported,

**Comparison of Protein Expression Profiles in Wild-Type and Hsf4tm1Xyk-Knockout Mice**

2-DE was used to identify differentially expressed lens proteins between the wild-type and Hsf4tm1Xyk-knockout mice, because SDS-PAGE did not demonstrate a significant difference between the homozygotes and heterozygotes. Protein spots with significant changes in densities (paired t-test, P < 0.05, n = 9) in a consistent direction (increase or decrease) were considered to be different. Analysis of the extracts which were separated by pH 3–10 IPG strips and 12.5% SDS-PAGE revealed that 15 protein spots were significantly different at expression levels between the wild-type and Hsf4tm1Xyk-knockout mouse lenses (Fig. 2A; Table 1). As shown in Figure 2A, most of lens proteins were distributed between pH 5–8 and 32 kDa. Thus, the lens extracts were further separated by pH 5–8 IPG strips and 15% SDS-PAGE. Another five proteins were found to be differently expressed between the wild-type and Hsf4tm1Xyk-knockout mouse lenses (Fig. 2B; Table 1). Of these spots, 9 were downregulated and 11 were upregulated in the Hsf4tm1Xyk-knockout mouse as shown in enlarged maps (Fig. 2C). The downregulated proteins were major structural proteins involving α- and β-crystallins, whereas the upregulated proteins were mainly enzymes and an intermediate filament protein, vimentin. An unexpected finding is that βB1 crystallins were both decreased and increased, in comparison with the wild-type control samples.

**Upregulation of Vimentin in the Hsf4tm1Xyk-Knockout Mouse Lens**

To confirm the upregulation of vimentin in the Hsf4tm1Xyk-knockout mouse identified by 2-DE, Western blot analysis was performed. The results confirmed the trends seen in 2-DE analysis for vimentin protein as shown in Figure 3A. There was approximately a threefold increase in the lenses of the Hsf4tm1Xyk-knockout mice. As vimentin may be a candidate target of HSF4, we further validated the expression of vimentin mRNA in the lens by Q-PCR analysis. An approximate fivefold increase in vimentin mRNA was observed in the Hsf4tm1Xyk-knockout mouse lenses. To exclude the possibility that the increased vimentin protein and mRNA accumulation occurs because they are more stable than other mRNAs and proteins, Western blot was also performed to detect the change of vimentin in early postnatal lens (PD1), when the lens was not obviously opaque. The results showed that the vimentin expression level was higher in the Hsf4tm1Xyk-knockout lens (Figs. 3C, 3D). Moreover, the expression of vimentin in the epithelial cells of the Hsf4tm1Xyk-knockout lens was markedly increased, as examined by immunohistochemical analysis (Fig. 4). To rule out the possibility that the increased vimentin expression is due to the aberrant lens fiber cell differentiation in general rather than specific regulatory effects on the genes, vimentin expression at earlier timepoints (embryonic day...
13.5, ED14.5, and PD1) was examined by immunohistochemical analysis (data not shown). The results showed that vimentin was upregulated from ED13.5 when lens and fiber cell morphology is intact. These results confirmed that vimentin expression level was upregulated in the lens of the Hsf4tm1Xyk-knockout mice.

Effect of HSF4 Inactivation on Denucleation in Lens Cells

As vimentin is involved in the denucleation process in lens fiber cells and previous work showed that denucleation in fiber cells of the Hsf4-null mice was inhibited,8,16 the denucleation process in the Hsf4tm1Xyk-knockout mouse lens at different developmental stages, including embryonic stages, was analyzed to identify the earliest point of divergence from the normal developmental track. Lenses were collected from ED15.5, PD1, and postnatal week (PW)4. Histologic analysis revealed that, at ED15.5, the lenses from the Hsf4tm1Xyk-knockout mice had normal features in the lens and fiber cell compartments (Figs. 5A, 5B). At PD1 the Hsf4tm1Xyk-knockout mouse lenses were slightly smaller than lenses from wild-type mice (Figs. 5C, 5D). The most striking phenotype in the
Hsf4tm1Xyk-knockout mouse lens was the failure of fiber cells to de-nucleate from PD1 (Figs. 5D, 5F). Reduced size, irregular shape, and disorganized structure with large vacuoles were other characteristics of the Hsf4tm1Xyk-knockout mouse lenses. Normally, lens epithelial cells differentiate into lens fiber cells and then lose their nuclei (Figs. 5C, 5E). In the lenses of the

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* Peptide counts matched in MS analysis.
† Cov, coverage by the matched peptides.
‡ The scores of proteins identified by MASCOT analysis (Matrix Science, Boston, MA; http://www.matrixscience.com).

**FIGURE 3.** Upregulation of vimentin in Hsf4tm1Xyk-knockout mice. (A) Protein expression of vimentin in the lenses of Hsf4tm1Xyk-knockout mice aged 8 weeks was significantly upregulated in comparison with levels in wild-type mice at the same age. (B) Comparison of mRNA expression of vimentin in the lenses of wild-type and Hsf4tm1Xyk-knockout mice aged 8 weeks. The mRNA level of vimentin in Hsf4tm1Xyk-knockout mice was significantly upregulated. (C) Protein expression of vimentin in the lenses of Hsf4tm1Xyk-knockout mice at PD1 in comparison with wild-type mice at the same age. (D) Comparison of mRNA expression of vimentin in the lenses of wild-type and Hsf4tm1Xyk-knockout mice aged PD1. *P < 0.01.
**Repression of Vimentin Gene Expression by HSF4**

To clarify whether HSF4 regulates vimentin gene expression, we performed EMSA. Based on the comparison of the sequence of vimentin promoter for potential HSF4-binding elements (HSEs) with the consensus sequence nGAAn, a putative HSF4-binding element (HSE-like) was identified in the vimentin promoter region (Fig. 6A). Because of the low endogenous expression of HSF4 in the mouse lens, a pcDNA3.1-HSF4b-expressing plasmid was transfected into SRA01/04 cells to amplify the signal for EMSA in which probes were labeled by biotin. As demonstrated in Figure 6B, the nuclear extracts of HSF4b-overexpressing cells significantly bound to wild-type HSE-like probe (Fig. 6B, lane 2) and this binding ability was completely blocked by the addition of a 400-fold molar excess of unlabeled HSE-like probe (cold probe; Fig. 6B, lane 3). When a similar excess of mutated probe was added, this binding ability could not be blocked (Fig. 6B, lane 4). Furthermore, HSF4b was identified in the HSE-vimentin/HSF4b complex by supershift analysis with anti-HSF4b antibody (Fig. 6B, lane 5). Moreover, ChIP was used to analyze the binding ability of HSF4 to vimentin promoter and, as shown in Figure 6C, HSF4 binding to vimentin promoter was confirmed. These results indicated that HSF4b had DNA-binding ability to the vimentin promoter.

To further confirm whether HSF4 can repress the transcription of vimentin, vimentin promoter-driven luciferase activity (VimPro-Luc) was analyzed. SRA01/04 cells with endogenous expression of HSF4 were cotransfected with HSF4b expressing vectors together with pGL3-VimPro-Luc plasmids. The results showed that, in the presence of HSF4b expression, VimPro-Luc expression was significantly antagonized by HSF4b in a dose-dependent manner (Fig. 7). These results indicated that HSF4 represses vimentin expression by directly binding to vimentin promoter.

**DISCUSSION**

Accumulated evidence indicates that HSF4 is an essential transcriptional regulator in lens development. Mutations of HSF4 have been detected in human families with defects in lens development and cataract formation. In the present study, Hsf4tm1Xyk knockout mice have been shown to undergo...
impaired lens development and subsequently to develop cataracts. Histologic analysis has confirmed the defect in the de-
nucleation process of lens fiber cells in Hsf4tm1Xyk
-knockout mice, reported previously.8,16 In addition, we demonstrated
that HSF4 could repress vimentin expression by directly bind-
ing to the vimentin promoter. This result provides a molecular
target for HSF4 to play a critical role in lens development. Such
findings shed light on the molecular mechanism for abnormal
lens development and cataract formation in
Hsf4tm1Xyk
-knockout mice, as well as in patients with mutations in HSF4.

Hsf4tm1Xyk
-knockout mice were used as a model in the present
study. The observation that the loss of HSF4 function
results in cataract was in agreement with previous reports.8,16
In addition, Hsf4tm1Xyk
-knockout mice showed other sequelae
such as iritis and exudates in anterior chamber. These sequelae
may represent secondary effects of cataract formation because
ruptured lenses were generally noted from the second week
after birth in Hsf4^gmm1Xyk
-knockout mice.

In understanding the molecular basis of cataract formation
caued by the loss of HSF4 function, it is important to identify
the target genes of HSF4. In a study of expression of single
candidate gene, Fujimoto et al.7 demonstrated that expressions
of FGF-1, -4, and -7 were increased in Hsf4-null lens. However,
it is not clear how HSF4 mediates a complex array of genes.
DNA microarray analysis showed that only a small group of
genes involved in lens development were differentially ex-
pressed between Hsf4-null mice and wild-type mice. Since
proteins are the functional output of gene expression, the
protein expression profile was examined in a proteomics ap-
proach and compared between the wild-type mice and
Hsf4^gmm1Xyk
-knockout mice in the present study. 2-DE electro-
phoresis combined with MALDI-TOF/TOF (tandem MS) revealed
an important molecular event in this model: the persistent high
expression of vimentin, which is a direct target of HSF4.

According to the 2-DE results, the differentially expressed
proteins can be classified into two groups: the downregulated
proteins and the upregulated proteins in the Hsf4^gmm1Xyk
-knockout mice when compared with those in the wild-type mice.
Further analysis revealed that the proteins in the downregu-
lated group were mainly structural proteins including αA-, αB-
and βB1-crystallins. Among them, the αA-, αB-, and βB-crystal-
lins are very strongly expressed in normal lens fiber cells.28–30
It is possible that the downregulation of these proteins con-
tributes to the severity of cataract and the smaller lenses at the
early stage of lens development. An interesting observation is
that both downregulated and upregulated βB1-crystallins were
observed in the Hsf4^gmm1Xyk
-knockout sample, compared with
the wild-type mouse lenses. A total of three downregulated
βB1-crystallin spots were identified in the Hsf4tm1Xyk
-knockout mouse lenses, and they may suggest different rates of
degradation or posttranslational modification or lens crystallin
expressions of candidate genes were also analyzed in SRA01/04
cells by ChIP analysis. PCR amplification of eluted DNA was
performed with specific primers for the
vimentin promoter region.

FIGURE 6. Interaction between HSF4b and vimentin promoter. (A)
Sequences of putative HSF4 binding elements in the vimentin pro-
moter. (B) EMSA competition experiments. Lane 1: the mobility of the
labeled wild-type probe without nuclear extracts; lane 2: the mobility of the
labeled wild-type probe with nuclear extracts in the absence of
a competitor; lane 3: sample with a 400-fold molar excess of unlabeled
HSE-like probe (cold probe), competed successfully with the wild-type
probe, which was used as specificity control; lane 4: a similar excess
of mutated probe, which does not bind to HSF4 (also as a specificity
control); lane 5: supershift assays with wild-type probe and nuclear
extracts in the presence of anti-HSF4 antibody. (C) Binding of HSF4 to
vimentin promoter in SRA01/04 cells by ChIP analysis. PCR amplification
of eluted DNA was performed with specific primers for the
vimentin promoter region.

FIGURE 7. Inhibition of vimentin promoter activity by HSF4b.
SRA01/04 cells were transfected with the indicated doses of
pcDNA3.1-HSF4b plasmids with 250 ng of pGL3-VimHSE-Luc plasmids
and pRL-TK Renilla. Cells were collected and lysed 24 hours after
transfection. The relative HSE-Luc activity was normalized by pRL-TK
Renilla. *P < 0.01, compared with HSF4 at 0 ng. Luc: luciferase.
isofoms in lenses between the two genotypes. On the other hand, the upregulated proteins were mainly enzymes and an intermediate filament protein. Since the metabolic activity in the fully differentiated fiber cells is lower due to the loss of nuclei and other subcellular organelles,\(^{31}\) the upregulated enzymes expressed in the Hsf4\(^{tm1Xyk}\)-null mouse lens may indicate the delayed lens cell differentiation, or a compensation for the defects in such lens fibers. Of note, among the upregulated proteins, proper levels of vimentin may be important in normal lens development, especially the denucleation of lens fiber cells.\(^{15}\)

It has been well documented that lens fiber cell differentiation is characterized by distinct morphologic and molecular changes: Epithelial cells exit from the cell cycle, undergo extensive elongation, develop specialized cell junctions, accumulate fiber-specific proteins (\(\beta_1\), \(\gamma\)-crystallin, filensin), and eventually lose their nuclei and intracellular organelles.\(^{19}\) This process is directed by a variety of transcription factors such as Pax6, Sox1, and L-Maf and by humoral factors including FGF, IGF, and TGF.\(^{52–34}\) Another study revealed that the expression of FGF-1, -4, and -7 increases in Hsf4-null mice.\(^8\) However, expression levels of these proteins were undetectable. In agreement with our results, previous microarray analysis showed no difference of FGF expression in Hsf4-null mice.\(^{16}\)

Another possibility is that the protein level of FGF is below the detectable limitation of the 2-DE analysis in our study.

The importance of vimentin in lens development, especially in lens fiber cell differentiation, was indicated by the study of vimentin-overexpressing transgenic mice.\(^{20}\) In such mice, the nuclei persisted and were scattered throughout the lens cortex. The striking similarities in lens phenotypes between vimentin-overexpressing and Hsf4\(^{tm1Xyk}\)-null mouse lenses suggest the importance of vimentin in lens demucleation and the regulatory role of HSF4 to vimentin. In contrast with the wild-type mice, 2-DE data showed that vimentin in the lens was upregulated in Hsf4\(^{tm1Xyk}\)-null mice, which were further confirmed by Western blot, Q-PCR, and immunohistochemistry. Meanwhile, histology analysis demonstrated the defects in the differentiation of primary lens fiber cells from Hsf4\(^{tm1Xyk}\)-null mice, particularly in the denucleation process. These results suggested that HSF4-regulated low vimentin expression was crucial for the lens fibers to denucleate.

The next key question is whether vimentin is directly regulated by HSF4. A basic local alignment search tool (National Center for Biotechnology Information, Bethesda, MD) showed an HSE-like sequence to be located in the vimentin promoter. As expected, HSF4 has DNA-binding ability to the vimentin promoter, as evidenced by EMSA and the ChIP assay. Moreover, the vimentin promoter-driven luciferase activity is suppressed by HSF4 expression in a dose-dependent manner. All these results suggest that HSF4 binds to the vimentin promoter directly and inhibits vimentin expression. Such inhibition is one of the mechanisms to assure the normal lens development. In the Hsf4\(^{tm1Xyk}\)-null mice, the decreased HSF4 function lost its regulatory effect on vimentin expression. The resultant overexpression of vimentin impaired the lens fiber cell differentiation, especially the denucleation. The molecular events by which the excess vimentin caused abnormal lens fiber cell differentiation are not clear. It has been shown that in lens fiber cells, the nuclei are lost at a specific stage during differentiation.\(^{21,35,36}\) The mature lens fiber cells, like the erythrocytes, display a lack of nuclei and vimentin filaments.\(^{21,22}\) Studies have shown that the vimentin interlinks the nucleus to the plasma membrane of lens fiber cells.\(^{22}\) Further evidence for the linkage between the fiber cell nuclei and the vimentin network was found in a study on vimentin-overexpressing transgenic mice. The prolonged existence of a large number of 10-nm intermediate filaments resulted in the failure of fiber cells to lose their nuclei in differentiation.\(^{20}\) Therefore, it is reasonable to postulate that the tight connection of the nucleus to the plasma membrane by the extra vimentin filaments causes abnormality in lens development and cataract formation.

In conclusion, we have identified vimentin as a novel target of HSF4 in lenses, and propose a mechanism by which the mutation of HSF4 impairs lens development and causes cataract. In the normal lens, HSF4 inhibits vimentin gene expression by directly binding to its promoter region. The loss of HSF4 function results in an increase in vimentin expression, as in Hsf4\(^{tm1Xyk}\)-null mice. The relative high expression of vimentin impairs the denucleation process in the lens fiber cells. As a result, abnormal lens development occurs, leading to cataract formation, as seen in Hsf4-null mice, as well as in some patients. The HSF4 and vimentin axis appears to be a new target for developing anti-cataract drugs, especially for those cataracts caused by aberrations in HSF4 expression.

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