이학석사학위 논문

이차 수정체 섬유세포 분화 과정에서 Sorting nexin 5의 역할

Role of Snx5 in differentiation of secondary lens fiber cells

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II. Abstract

Role of Snx5 in differentiation of secondary lens fiber cells

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Sorting nexin 5 (Snx5) has been known as a member of retromer complex, required for recycling of proteins back to cell membrane through Trans-Golgi-Network. Snx5 has a PX (Phospoinositide-binding structural) domain in its N-terminal and a BAR (Bin, Amphiphysin, Rvs) domain in its C-terminal. In general, Snx5 binds to phosphatidylinositol-3-monophosphate (PtdIns3P) with its PX domain and makes curvature of membrane with BAR domain. Through this interaction, Snx5 helps a separation of vesicles from endosomal

compartment to pass through trans-golgi-network (TGN).

In lens development, reciprocal interactions between ligands and receptors play crucial roles. Indeed, several receptor-mediated events affect each process of lens development. Growth factors signalings are typical examples, such as bone morphogenetic protein (BMP), basic fibroblast growth factor (bFgf), and epidermal growth factor (Egf). However, it is largely unknown how those receptor levels are regulated.

Here, I show that the ablation of *Snx5*, a member of retromer complex, induces a cataract formation in lens of *Snx5*$^{-/-}$ mice. Histological analysis revealed that *Snx5*$^{-/-}$ mice have defective equatorial region of lens and abnormal differentiating secondary lens fiber. Also, the depletion of *Snx5* resulted in overexpression of *α-smooth muscle actin (α-SMA)* in equatorial region of lens. Taken together, I propose that *Snx5* gene plays an important role in trans-differentiation of lens fiber cells and cataract formation.

Key words: Sorting nexin 5, Snx5, Cataract, Lens, Secondary lens fiber cells

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III. Introduction

Lens development begins as the optic vesicles outgrow from the forebrain. After the evagination of optic vesicles, they come into very close apposition to the head surface ectoderm, thereby inducing the thickening of head surface ectoderm and the formation of lens placode. Subsequently, the lens placode invaginates and develops into a lens vesicle. The optic cup, meanwhile, forms the retina. Once the lens vesicle takes its shape, the posterior half of the vesicle elongates toward the anterior side becoming primary lens fiber cells. On the other hand, a monolayer of lens epithelial cells which is located in the anterior side of the lens (Lovicu and McAvoy, 2005; McAvoy et al., 1999) proliferates just above the equator region (Kallifatidis et al., 2011) and migrates toward the edge of the lens where it differentiates into secondary lens fiber cells (Mochizuki and Masai, 2014). Once the migration and differentiation starts, the proliferating and differentiating lens epithelial cells continuously form mature secondary lens fiber cells throughout life.

Continuous differentiation of secondary lens fiber is very important to maintain the transparent lens. Receptor–ligand interaction is the key regulator in the differentiation. Basic fibroblast growth factor (bFgf), which is derived from retina and exists in vitreous humor, is a representative example. During postnatal development, the low

dose of bFgf accelerates the proliferation of lens epithelial cells, while the high dose of bFgf induces the differentiation of lens fiber cells (McAvoy and Chamberlain, 1989).

However, in pathological conditions, lens epithelial cells are affected by transforming growth factor-β (Tgf-β) which is upregulated in vitreous humor. With Tgf-β, they do not differentiate into proper secondary lens fiber cells and instead undergo an epithelial-mesenchymal transition (EMT), eventually developing some signs of cataracts. Once EMT occurs, the lens epithelial cells change their cuboidal shapes into spindle-like shape that frequently expresses α-smooth muscle actin (α-SMA). In lens explant culture in the presence of Tgf-β, these transformed lens epithelial cells express α-SMA, which is commonly used as a marker for myofibroblast-like lens cells (Hales et al., 1995; Lee and Joo, 1999; Liu et al., 1994). In those kinds of receptor-ligand interaction in lens, a proper concentration of ligands and an appropriate recycling/degradation level of receptors are important. But it is barely known how those signals are regulated by recycling/degradation level of receptors.

Most transmembrane receptors undergo endosomal sorting pathway after ligand binding. In this pathway, the destinies of receptors in early endosomes are divided into two major circuits: lysosomal degradation pathway and recycling pathway. Between them, Sorting nexin (SNX) family proteins play a key role in retrograde process,

which is responsible for transporting proteins back to cell membrane through Trans-Golgi-Network(TGN). SNX5, a member of SNX family and retromer complex, has a PX (Phospoinositide-binding structural) domain in its N-terminal and a BAR (Bin, Amphiphysin, Rvs) domain in its C-terminal. In general, SNX5 binds to phosphatidylinositol-3-monophosphate (PtdIns3P) with its PX domain and makes curvature of membrane with BAR domain, allowing separation of post-TGN vesicles from endosome (Cullen, 2008; Cullen and Korswagen, 2012; Worby and Dixon, 2002).

The function of SNX5 was first reported as a putative binding partner of FANCA (Fanconi Anemia Complementation Group A) (Otsuki et al., 1999). SNX5 was also known as a trafficking molecule of Epidermal Growth Factor Receptor (EGFR) and Cation-Independent Mannose 6-Phosphate Receptor (CI-MPR). Recently, several papers reported that SNX5 interacts directly with phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P2) and phosphatidylinositol phosphate kinase gamma i5 (PIPKγi5) for endosomal sorting of EGFR (Sun et al., 2013). The CI-MPR-related SNX5 functions were first suggested that SNX5 forms retromer complex with SNX1 and SNX6 in HeLa cells and plays a role in endosome-to-TGN retrieval of the CI-MPR (Wassmer et al., 2007). In addition, SNX5 was co-immunoprecipitated with DOCK180 which is the archetype of the DOCK180-family guanine nucleotide

exchange factor (GEF), and was found that the interaction between SNX5 and DOCK180 is potentially involved in retromer-mediated retrogradation of CI-MPR. However, the precise physiological role of SNX5 in mammal has barely been elucidated until now because most of previous studies have been done in vitro system.

Here, I show that cataract formation in $Snx5^{-/-}$ mice is potentially caused by abnormal differentiation of lens epithelial cells into secondary lens fiber cells. In addition, trans-differentiated lens fiber cells express α-Sma in equatorial region of the lens, indicating the dysregulation of lens differentiation. Taken together, I suggest that the disruption of Snx5 gene have a significant impact on the differentiation of secondary lens fiber cells and eventually develops cataract formation.

IV. Materials and methods

IV-1. Mice

I used *Snx5*$^{-/-}$ mice which are previously reported (Im et al.). C57BL/6 mice in previous study are backcrossed with pure ICR strain for 6times and then used in this study. Mice used in this study were housed in the specific pathogen-free facility of Seoul National University, and all the experiments were performed in accordance with the guidelines of the animal ethics committee. All animal experiments were approved by the Seoul National University Institutional Animal Care and Use Committee (Approval number: SNU130327-9). In all experiments, all efforts were made to minimize suffering.

IV-2. X-gal staining

Whole eyeballs or sections of eyeballs were fixed in 2 % formaldehyde and 0.2 % glutaraldehyde in PBS for 2 hours at 4 ° C. For whole eyeballs, tissues were embedded in OCT Compound (Tissue Tek) following 3 times of washing, and then sectioned at 10 μm thick. The sectioned tissues were washed with X-gal washing buffer [2 mM $MgCl_2$, 0.02 % Nonidet P-40 (NP-40), and 0.05 % Deoxycholate in PBS] after 3 times of washing with PBS, and then

incubated in X-gal staining buffers [5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 1 mg/ml X-gal substrate in X-gal washing buffer] for overnight at 37 ° C. Stained images were taken with a Zeiss Axio Imager A2 microscope and a Diagnostic SPOTFlex camera.

IV-3. Histological analysis

After removing eyeballs from mice, eyeballs were processed with modifications of the standard method (Madakashira et al., 2012). The protocol for this step is kindly provided by Dr. Micheal L. Robinson. Dehydrated and paraffinized tissues were embedded in paraffin and then cut in 5 μm sections. After those procedures, the sections were used for hematoxylin and eosin (H&E) staining and immunofluorescent staining. Mouse anti-α-smooth muscle actin (1:1000; Abcam; #Ab7817-500), Alexa Fluor 594 goat anti-mouse (1:100; Molecular Probes; #A110326), and Hoechst 33342 (1:1000; Molecular probes; #H3570) were used for nucleic acid staining. All of these stained sections were taken as images with a Zeiss Axio Imager A2 microscope and a Diagnostic SPOTFlex camera.

IV-4. Quantitative real time PCR analysis

Total RNA extraction, reverse transcription (RT-PCR), and

quantitative real time PCR (qRT-PCR) were performed as previously described (Im et al., 2013). The sequences of the primers used in this study were as follows.

*mSnx5*_F: 5' -ggagatgtttggaggcttttt-3'

*mSnx5*_R: 5' -caggaatccttgatcctgttg-3'

*mCryga*_F: 5' -actacaggcgctaccacgac-3'

*mCryga*_R: 5' -gccaggaacacaggttgatt-3'

*mCrygb*_F: 5' -gtttcagcgactccattcgt-3'

*mCrygb*_R: 5' -gtggaagcgatcctgaagag-3'

*mCrygc*_F: 5' -ggccaccagtacttcctgag-3'

*mCrygc*_R: 5' -tctcatacagccgcattctg-3'

mCrygd_F: 5'-aacctgcagccctacttcag-3'

mCrygd_R: 5'-aacccatccactgctggtag-3'

mCryge_F: 5'-accctgactaccagcagtgg-3'

mCryge_R: 5'-gcagtcgtctgtgatctcca-3'

mCrygf_F: 5'-accctgactaccagcagtgg-3'

mCrygf_R: 5'-gcagtcgtctgtgatctcca-3'

*mCrygs*_F: 5' -tcgtacctaagtcgctgcaa-3'

*mCrygs*_R: 5' -atccaacgctggtattcagg-3'

IV-5. Statistical analysis

In this study, all quantitative graphs are expressed as mean ± standard error of the mean (SEM) or Standard Deviation (SD). p-values were calculated by the Student's t-test. A p-value $\leq$ 0.05 was considered to be statistically significant.

V. Results

V-1. Cataract development in *Snx5*$^{-/-}$ mice

More than 50% of *Snx5*$^{-/-}$ mice are lethal before weaning and survived mice showed growth retardation (Im et al., 2013). Intriguingly, the opaque region in the eyes were frequently observed in 2-month-old *Snx5*$^{-/-}$ mice (Fig. 1A). This white cloudy area was detectable only with slit lamp biomicroscopy and to naked eyes (Fig. 1B, C) at 3- and6-week-old *Snx5*$^{-/-}$ mice, respectively. The opaque area restricted mostly in lens but not cornea strongly indicates cataract formation in *Snx5*$^{-/-}$ mice.

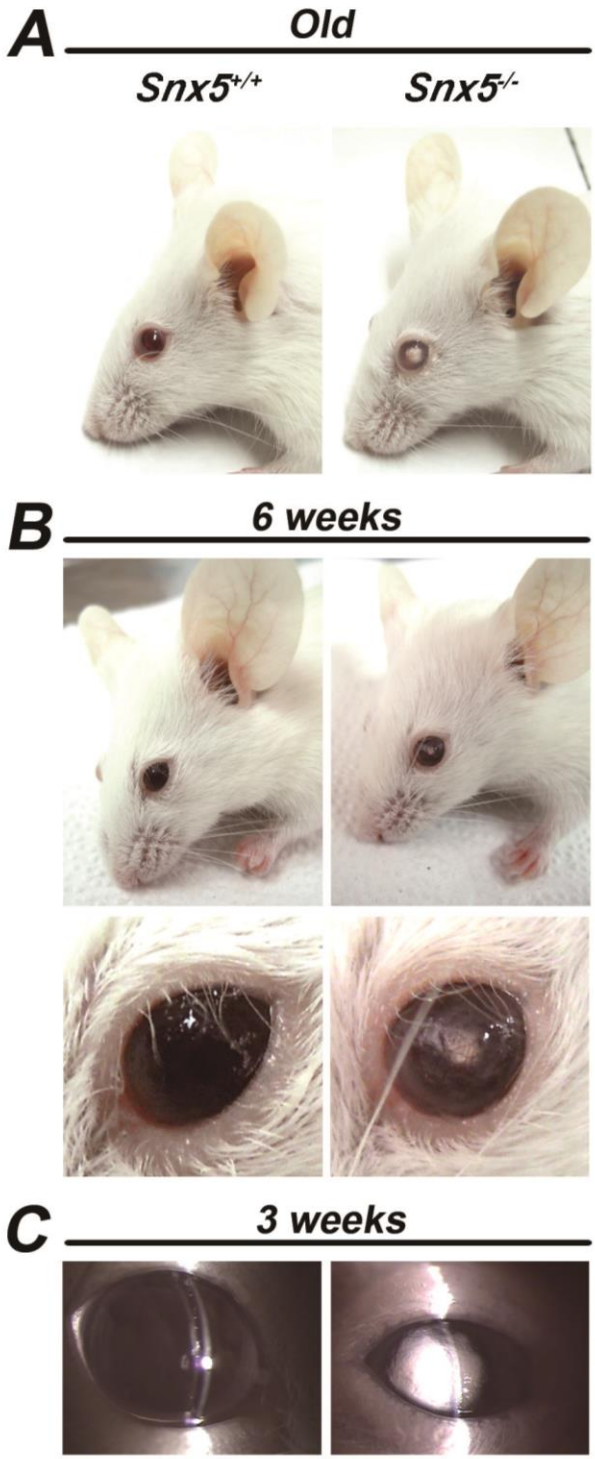
A
Old
Snx5+/+
Snx5-/-
B
6 weeks
C
3 weeks

Figure 1. Cataract development in *Snx5*$^{-/-}$ mice

(A–B) Opaque eyes of *Snx5*$^{-/-}$ mice are visible to naked eyes after 6 weeks of age.

(C) Opaque lens is remarkably detectable with slit lamp biomicroscopy in 3–week–old *Snx5*$^{-/-}$ mice.

V-2. Expression pattern of *Snx5* in lens

To investigate whether Snx5 is implicated in cataract development in *Snx5*$^{-/-}$, the expression pattern of *Snx5* in lens was examined. Since the *Snx5*$^{+/-}$ mice have the β-geo cassette between exon 7 and exon 8, the *Snx5*-expressing cells were stained with X-gal. Whole mount X-gal staining of 4-week-old lens showed high expressions of *Snx5* at the edge of the lens (Fig. 2A). Similar expression patterns were also observed in 2-week-old and postnatal day 0 (P0) lens (Fig. 2B, C). In P0 lens, β-gal activity was highly observed in epithelial cells and equatorial fiber cells of the lens (Fig. 2C'). Collectively, the restricted expression of *Snx5* in epithelial cells and equatorial fiber cells (Fig. 2D) suggests that *Snx5* is implicated in the differentiation of secondary lens fiber throughout lifetime, which occurs during lifetime since embryonic day (E) 12.5 (Kallifatidis et al., 2011).

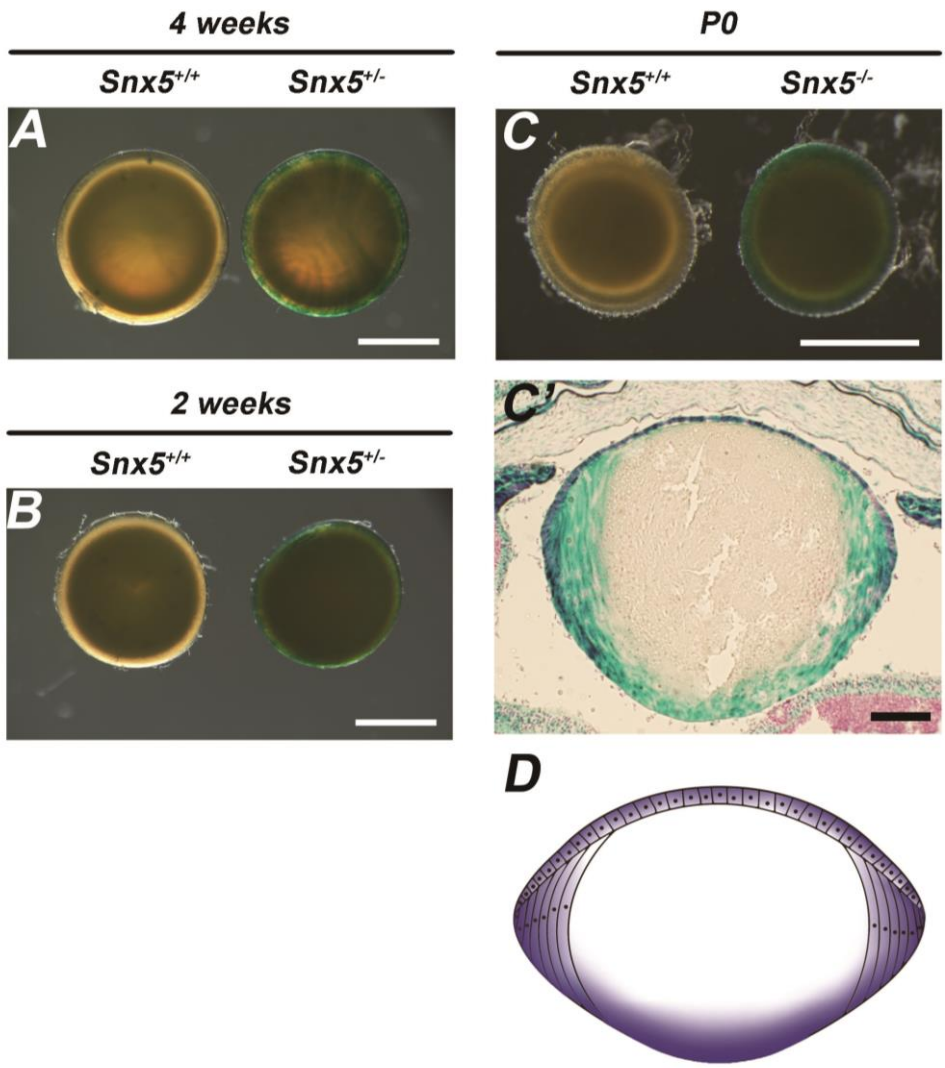
4 weeks
Snx5+/+
Snx5+/-
A
2 weeks
Snx5+/+
Snx5+/-
B
P0
Snx5+/+
Snx5-/-
C
C'
D

Figure 2. Expression pattern of Snx5 in lens

(A–C) Whole mount X–gal staining of *Snx5*$^{+/+}$, *Snx5*$^{+/-}$ and *Snx5*$^{-/-}$ lenses. β–gal activity was highly detected at the edge of *Snx5*$^{+/-}$ and *Snx5*$^{-/-}$ lenses. (Scale bar: 1 mm)

(C') Section staining of P0 *Snx5*$^{-/-}$ lens. β–gal activity was highly observed in epithelial cells and equatorial and posterior lens fiber cells of *Snx5*$^{-/-}$ lenses. (Scale bar: 100μm)

(D) Schematic view of expression pattern of *Snx5* in lens.

V-3. Disorganized structure of fibers in the equatorial region of *Snx5*$^{-/-}$ lens

The morphological change of *Snx5*$^{-/-}$ lens was analyzed histologically (Fig. 3A-D). In the E15.5 embryo, the primary fiber cells in *Snx5*$^{-/-}$ lens were morphologically indistinguishable from *Snx5*$^{+/+}$ lens (Fig. 3A), indicating that the disruption of *Snx5* gene did not affect the development of primary lens fiber (Fig. 3 A). Although 4-week-old *Snx5*$^{-/-}$ lens fibers retained normal architecture in the transitional zone, they appeared abnormal in central region (Fig. 3B). By 6 weeks of age, *Snx5*$^{-/-}$ lenses showed the vacuoles in the transitional zone and the disorganized fiber cells in the posterior region (Fig. 3C, C'), suggesting abnormal secondary lens fiber cell differentiation in *Snx5*$^{-/-}$ mice .

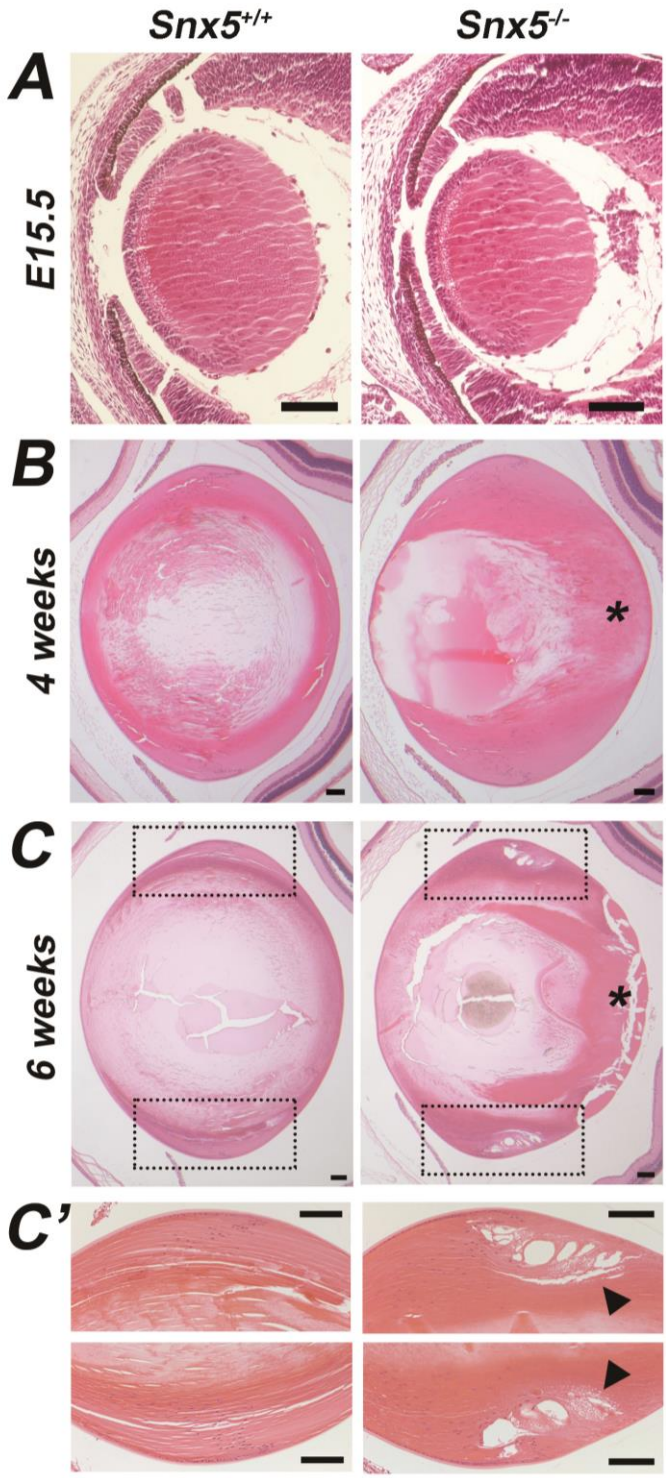
Snx5+/+
Snx5-/-
A
E15.5
B
4 weeks
*
C
6 weeks
*
C'

Figure 3. Disorganized structure of fibers in the equatorial region of *Snx5*$^{-/-}$ lens

(A–D) Cross sections of lenses at E15.5 (A), 4–(B), and 6–week–old (C) were stained with H&E. Note the change of lens morphology and the migration of lens fiber cells toward anterior side of lens in *Snx5*$^{-/-}$ lens, compared with *Snx5*$^{+/+}$ lens. C' is higher magnifications of the squared areas in C.

(A) E15.5 *Snx5*$^{-/-}$ lens shows comparable morphology to *Snx5*$^{+/+}$ lens. (Scale bar: 1mm)

(B) 4–week–old *Snx5*$^{-/-}$ lens shows abnormal migration of lens nucleus toward anterior region of the lens. (Scale bar: 1mm)

(C) 6–week–old *Snx5*$^{-/-}$ lens shows severely disorganized lens structure, aberrant morphology of lens nuclear , and vacuoles in the equatorial region(Scale bar: 1mm).

(C') Higher magnifications of the squared areas in C.

(Asterisks: abnormal posterior side of lens nucleus, Arrowhead: vacuole formation)

V-4. Reduced expression of differentiation markers in *Snx5*$^{-/-}$ lens

To determine whether the *Snx5*$^{-/-}$ lenses have normally differentiated lens fiber cells, qRT-PCR was performed to investigate mRNA expression levels of *γ-Crystallins*, markers of differentiated secondary lens fiber cells (Andley, 2007). At P0 mice, *γ-Crystallins* expression levels of *Snx5*$^{-/-}$ lenses was comparable to those of *Snx5*$^{+/+}$ lenses. However, consistent with morphological changes shown in equatorial region of *Snx5*$^{-/-}$ lenses (Fig. 3), *γ-Crystallins* expression levels of *Snx5*$^{-/-}$ lenses were significantly lower in 2-, 4-, and 6-week-old *Snx5*$^{-/-}$ mice(Fig. 4 A-D). Together, these results suggest that *Snx5*$^{-/-}$ lenses have abnormally differentiated secondary lens fiber cells.

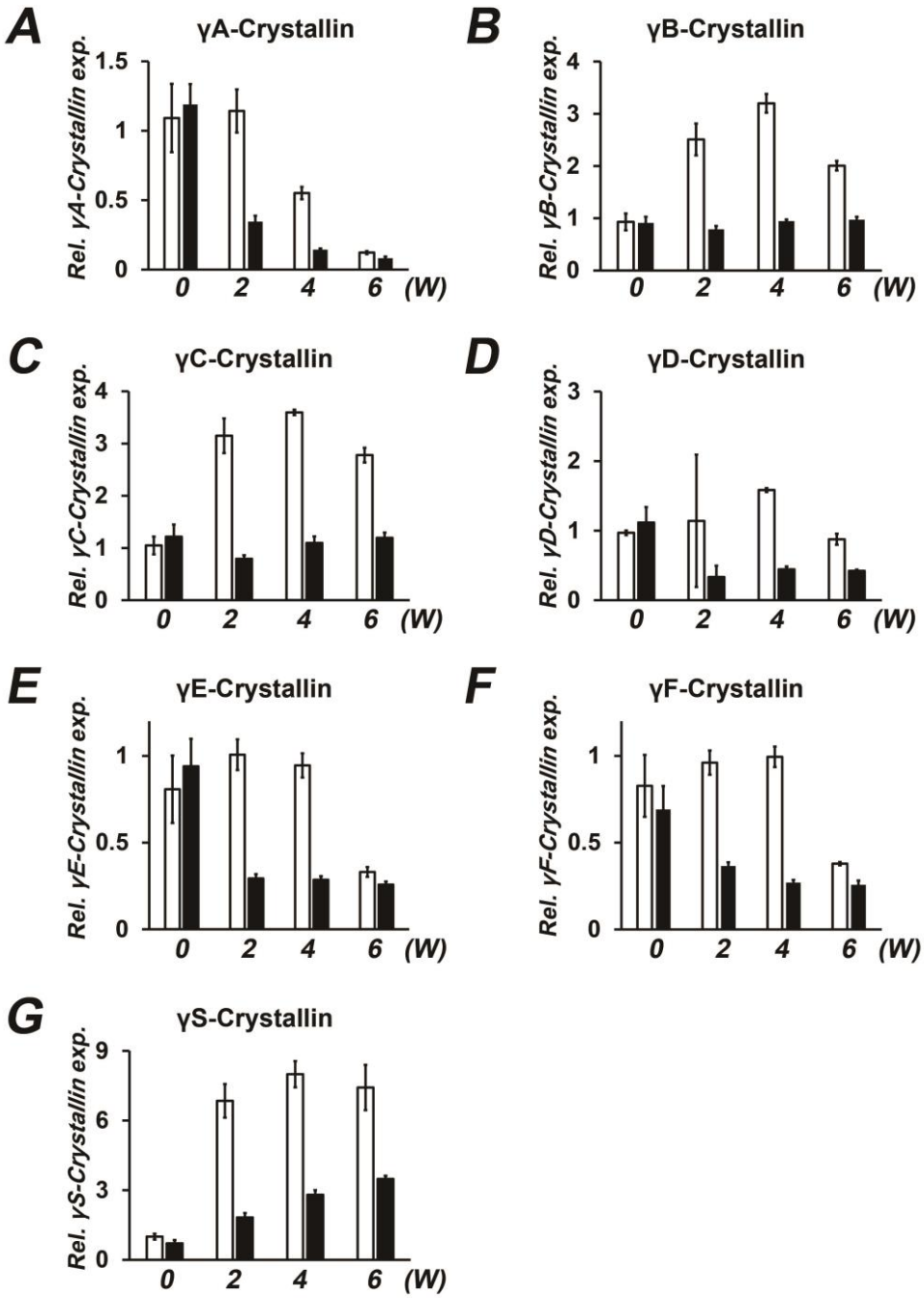
A
γA-Crystallin
Rel. γA-Crystallin exp.
1.5
1
0.5
0
0
2
4
6
(W)
B
γB-Crystallin
Rel. γB-Crystallin exp.
4
3
2
1
0
0
2
4
6
(W)
C
γC-Crystallin
Rel. γC-Crystallin exp.
4
3
2
1
0
0
2
4
6
(W)
D
γD-Crystallin
Rel. γD-Crystallin exp.
3
2
1
0
0
2
4
6
(W)
E
γE-Crystallin
Rel. γE-Crystallin exp.
1
0.5
0
0
2
4
6
(W)
F
γF-Crystallin
Rel. γF-Crystallin exp.
1
0.5
0
0
2
4
6
(W)
G
γS-Crystallin
Rel. γS-Crystallin exp.
9
6
3
0
0
2
4
6
(W)

Figure 4. Reduced expression of differentiation markers in *Snx5*$^{-/-}$ mice

(A-G) Expression of *γ-Crystallins*, which are known as differentiation marker genes, was assessed by qRT-PCR. The expression level of *γ-Crystallins* were reduced in 2-, 4-, and 6-week old lenses. Gene expression was normalized to β-actin mRNA level in each sample. (P0, 2-week-old mice: n=3 and 4-, 6-week old mice: n=2)

V-5. Aggregation of myofibroblast-like cells in lens bow region

In pathological conditions, epithelial cells trans-differentiate into mesenchyme-like cells but not into normal secondary lens fiber cells. Subsequently, these abnormal cells accumulate around the lens capsule (Lovicu et al., 2004). I examined whether this kind of trans-differentiation occurred in equatorial region where differentiation starts. The expression level of α-SMA significantly increased in the lens of *Snx5*$^{-/-}$ mice although it was barely expressed in those of *Snx5*$^{+/+}$ mice. (Fig. 5A-C) I confirmed that the increased expression level of *α-SMA* in *Snx5*$^{-/-}$ mice with qRT-PCR, showing that both mRNA and protein level of α-SMA are increased in *Snx5*$^{-/-}$ mice. Since lens epithelial cells are induced to differentiate in the equatorial region, the defects in *Snx5*$^{-/-}$ mice indicate that there is dysregulation in differentiation of the secondary lens fiber. These results also indicate that trans-differentiation into α-SMA-positive cells occurred in bow region of *Snx5*$^{-/-}$ lens.

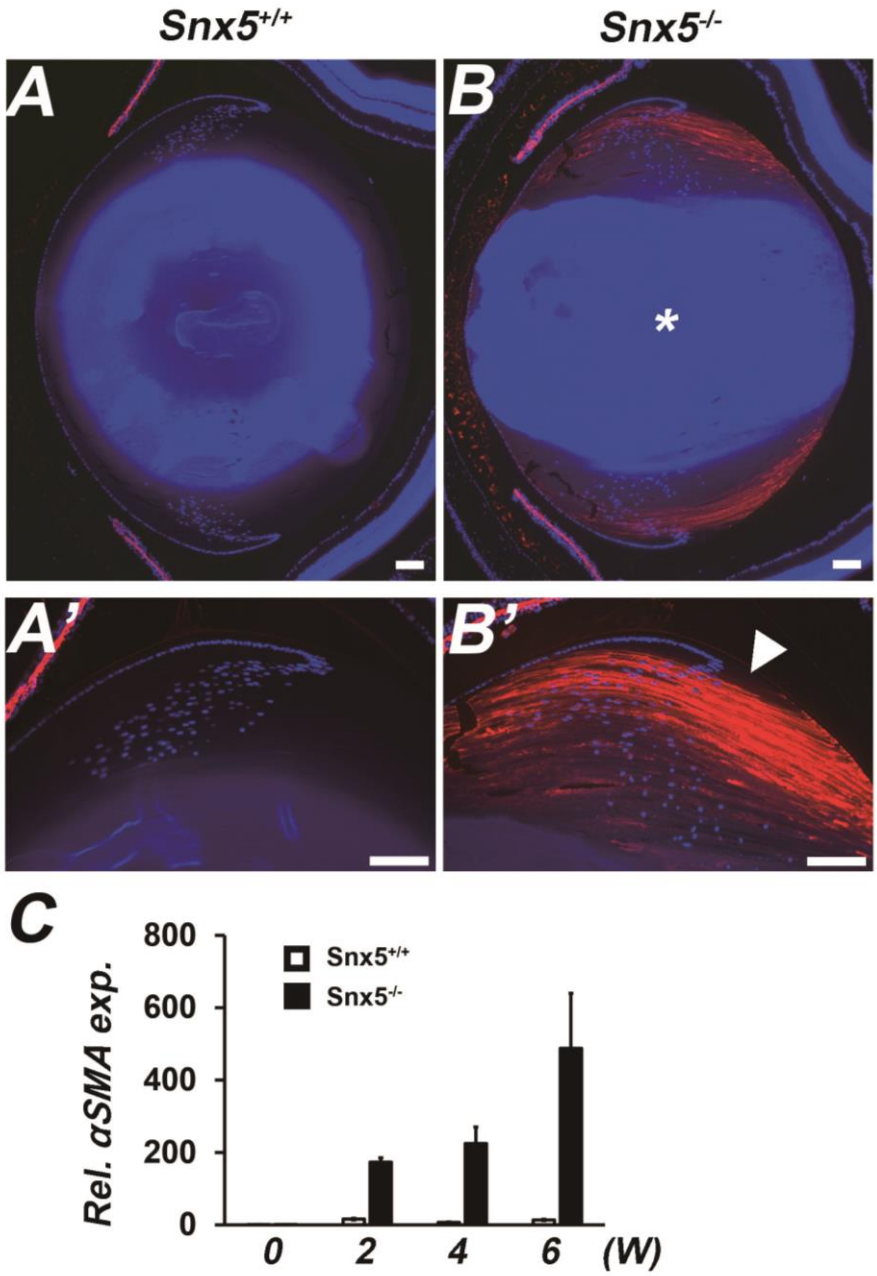
Snx5+/+
Snx5-/-
A
B
*
A'
B'
C
Rel. αSMA exp.
800
600
400
200
0
Snx5+/+
Snx5-/-
0
2
4
6
(W)

Figure 5. Aggregation of myofibroblast-like cells in lens bow region

(A) *Snx5*$^{-/-}$ mice show spindle-shaped α-SMA-positive cells in equatorial region. Also, lens nuclear was stained with Hoechst antibody in *Snx5*$^{-/-}$ mice, compared to *Snx5*$^{+/+}$ mice. (Scale bar: 100μm)

(B) Consistently with (A), expression level of *α-SMA* is significantly higher in *Snx5*$^{-/-}$ lenses. These expression level increased by age. Gene expression was normalized to β-actin mRNA level in each sample. (P0, 2-week-old mice: n=3 and 4-,6-week old mice: n=2)

VI. Discussion

Here, I found that Snx5 plays a key role in differentiation of secondary lens fiber cells. Targeted disruption of *Snx5* gene developed cataract caused by abnormal differentiation of lens epithelial cells into myofibroblast-like cells. Since morphology and *γ-crystallin* expression of *Snx5*$^{-/-}$ lens are normal at embryonic day (E) 15.5 and P0, respectively, I concluded that the cataract is developed by defective secondary lens fiber cells not by primary lens fiber cells.

Intriguingly, the morphological changes such as formation of vacuoles and accumulation of abnormal α-SMA-positive cells were prominent in the equatorial region of *Snx5*$^{-/-}$ lenses, indicating EMT occurred during the differentiation of secondary lens fiber cells in the bow region. In addition to the formation of fibroblast-like cells, those abnormal lens fiber cells seem to migrate toward a more posterior region of the lens. Consequently, according to histological analysis, the center of lens nucleus is skewed toward a more anterior region of lens due to the accumulation of fibroblast-like cells in the posterior region. In addition, Hoechst-stained nuclei are observed in the center of *Snx5*$^{-/-}$ lens while none are observed in normal lens, indicating the defects in differentiation process of lens fiber cells. Also, decreased expression of *γ-Crystallins* further supports that lens fiber cells in *Snx5*$^{-/-}$ mice undergo abnormal differentiation.

Among several processes of lens development, the lifelong differentiation of lens epithelial cells into secondary lens fiber cells is highly related to progressive cataract. Growth factors such as Fgf and insulin-like growth factor (Igf) are well known molecules to induce and regulate the differentiation of lens fiber cells during early lens development (Beebe et al., 1980; Beebe et al., 1987). According to previous studies about ligand-receptor interaction-mediated regulations, it is possible that Snx5, a retromer complex protein, controls the levels of cell surface receptors and consequently regulates the differentiation of lens fiber cells.

Tgfβ signaling is well known as an EMT inducing factor in differentiation of secondary lens fiber cells. Many studies have been reported that there is a cataractous change in cultured whole rat lens and explanted lens epithelial cells of rat with Tgfβ treatment (Hales et al., 1995; Liu et al., 1994). In accordance with *ex vivo* study, *Tgfβ1* transgenic mice showed fibrotic plaques with increased levels of α-SMA around the anterior capsule of lens by P21 (Srinivasan et al., 1998). Also, atypical expressions of α-SMA in fibroblast-like cells are used as a marker for posterior capsular cataract (PCO) in human patients. Interestingly, consistent with *ex vivo,* mouse and human models of EMT, the *Snx5*$^{-/-}$ mice also showed α-SMA-positive cells around the lens capsule and characteristics of cataract in their lenses. Furthermore, the recent papers about interactions between EGFR

and SNX5 suggest that SNX5 helps sorting of EGFR onto the intraluminal vesicles (ILVs) of the multi-vesicular body (MVB) in normal conditions (Sun et al., 2013). Therefore, I speculated that if Snx5 is related to the degradation of Tgfβ receptors as known to precede the degradation of EGFR, it is possible that the disruption of *Snx5* gene causes the overexpression of Tgfβ target genes, and the subsequent occurrence of unnecessary EMT in the lens fiber cells. Together, previous studies and this study indicate that Snx5 regulates the degradation of Egfr and thus, the disruption of *Snx5* gene can enhance the Egfr signaling, leading to cataractous phenotype.

There are several human family case reports about congenital cataract with genetic mapping of candidate genes related to congenital cataract formation. Interestingly, there were no noticeable mutations in candidate gene such as Bfsp1 (Li et al., 2006; Yamada et al., 2000; Zhang et al., 2008). However, with locus information of *SNX5* gene in human, I strongly suggest that *SNX5* is a new candidate gene for reported family cases. If it is the case, *Snx5*$^{-/-}$ mice, which have mutation on *SNX5* gene, used in this study can be an effective model for human cataract clinical research.

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VIII. 국문 초록 (Abstract in Korean)

이차 수정체 섬유세포 분화 과정에서

Sorting nexin 5의 역할

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Sorting nexin 5 (Snx5)는 세포막 단백질의 recycling/degradation을 조절하는 레트로머 복합체의 구성 요소이다. Snx5의 N-말단부에는 엔도좀의 막에 직접 결합할 수 있는 PX (Phosphoinositide-binding structural) 도메인을, C-말단부에는 엔도좀의 막에 커브를 형성할 수 있도록 하는 BAR (Bin, Amphiphysin, Rvs) 도메인을 갖고 있다. 이러한 구조를 통해 엔도좀으로부터 형성되는 소포가 따로 떨어져나올 수 있도록 돕는다.

수정체 발생 과정에서 리간드-수용체 신호가 중요한 역할을 한다. 이와 같은 리간드-수용체 신호가 전달되는 정도를 조절할 수 있는 방법은 크게 두 가지가 있다. 리간드 레벨을 조절하는 경우와 세포막에

존재하는 수용체의 레벨을 조절하는 경우이다. 수정체 발생 과정과 관련하여 다양한 리간드-수용체 신호에 대한 연구가 활발하게 이루어져 왔음에도 불구하고, 아직까지 이러한 리간드-수용체 신호들의 조절 기작은 명확하게 밝혀지지 않은 부분이 많다. Snx5는 레트로머 복합체를 구성하는 요소 중의 하나로써, 수용체의 레벨을 조절하는데 기여할 수 있다.

이러한 Snx5 유전자를 결손 시킨 마우스에서 백내장 표현형을 관찰했다. 조직학적 분석을 통해 *Snx5*$^{-/-}$ 마우스에서 E15.5 시기 까지는 대조군 마우스와 차이가 없음을 확인하였고, 그 이후인 4주 이후부터 대조군 마우스와 형태학적으로 차이가 나는 것을 관찰하였다. 이를 통해 *Snx5*$^{-/-}$ 마우스의 일차 수정체 섬유세포 발달까지는 이상이 없고, 이차 수정체 섬유 세포의 이상일 것이라 유추할 수 있었다. 이차 수정체 섬유 세포의 표지인 감마 크리스탈린의 발현 레벨을 확인했을 때, *Snx5*$^{-/-}$ 마우스에서 확연히 감소되어 있는 것을 통해 *Snx5*$^{-/-}$ 마우스에서 정상적으로 이차 수정체 섬유 세포가 형성되지 않았음을 유추할 수 있었다. 또한, 분화가 일어나는 수정체 적도 부분에 비정상적인 α-SMA (α-Smooth muscle actin)를 발현하는 세포들을 관찰함으로써, *Snx5*$^{-/-}$ 마우스에서는 이차 수정체 섬유 세포로 정상적인 분화가 일어나지 않고, 다른 방향인 myofibroblast-like cell로 분화가 일어났음을 알 수 있었다.

핵심어: Sorting nexin 5, Snx5, 백내장, 수정체, 이차 수정체 섬유 세포

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