의학석사 학위논문

Localization of primary cilia in mouse retina

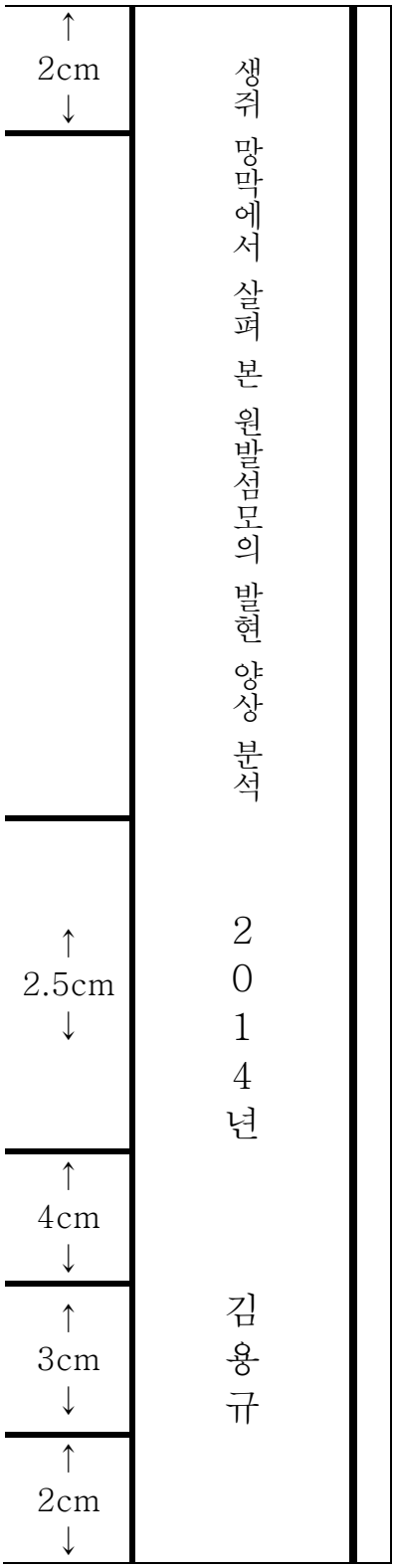
생쥐 망막에서 살펴 본 원발섬모의 발현 양상 분석

2014년 2월

서울대학교 대학원

의학과 안과학

김 용 규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Localization of primary cilia in mouse retina

지도 교수 유 영 석

이 논문을 의학석사 학위논문으로 제출함
2013년 10월

서울대학교 대학원
의학과 안과학
김 용 규

김용규의 의학석사 학위논문을 인준함
2013년 12월

위 원 장 박 규 형 (인)
부위원장 유 영 석 (인)
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학위논문 원문제공 서비스에 대한 동의서

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1. 동의사항
① 본인의 논문을 보존이나 인터넷 등을 통한 온라인 서비스 목적으로 복제할 경우 저작물의 내용을 변경하지 않는 범위 내에서의 복제를 허용합니다.

② 본인의 논문을 디지털화하여 인터넷 등 정보통신망을 통한 논문의 일부 또는 전부의 복제.배포 및 전송 시 무료로 제공하는 것에 동의합니다.

2. 개인(저작자)의 의무
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② 서울대학교는 본 논문에 대한 공개의 유보나 해지 신청 시 즉시 처리해야 합니다.

논문제목 : Localization of primary cilia in mouse retina

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제 출 일: 2013 년 10 월 17 일

서울대학교총장 귀하

Abstract

Introduction: The primary cilia are considered as a “cellular antennae” that sensing and interchanging the information with its extracellular environment and nearly all mammalian cells have a single primary cilium. In the retina, the outer segment (OS) and connecting cilium of photoreceptor is known to be a specialized form of primary cilia, but studies on cilia in other layer of retina is scarce. In this study, we aimed to find out the expression of primary cilia using different ciliary markers in a whole layer of mouse retina.

Methods: C57BL/6 mice were sacrificed on postnatal 26th day and eyes were enucleated from it. The eyes were embedded in paraffin, sectioned to 4㎛ thickness and stained with three different ciliary markers; Arl13b, Acetylated α tubulin and Adenylyl cyclase III. Using immunofluorescence, we evaluated the expression and relative location of these markers in the retina.

Results: Arl13b was expressed in photoreceptor layer (PRL), outer plexiform layer (OPL) and weakly in inner plexiform layer (IPL). Adenylyl cyclase III showed similar patterns with Arl13b and it also showed diffuse reactivity in ganglion cell layer (GCL). Acetylated α tubulin was expressed in PRL, OPL, IPL and also some longitudinal staining patterns were observed throughout the whole thickness which is thought to be the Muller cells. Arl13b and

Adenylyl cyclase III showed diffuse staining in inner segment of PRL while Acetylated α tubulin showed speckled patterns in connecting cilium area. In OPL, all three markers showed strong reactivity with feathery patterns along the neuronal synapses. However, we couldn't find any single strand-like shapes that emerge directly from the cell surfaces.

Conclusions: In immunofluorescence with three different ciliary markers in mouse retina, we found positive reaction in PRL, OPL and GCL. We couldn't directly prove the strand-like shape of cilia in those areas, which might more specifically suggest the presence of cilia, but our results suggest the possibility of presence of primary cilia in OPL and GCL in addition to PRL OS. Furthermore, in the outer plexiform layer, all three markers showed intense staining along the neuronal synapses, which suggests that the neuronal processes themselves might share the features of cilia.

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Keywords: Primary Cilia; Mouse retina; Arl13b; Acetylated α tubulin; Adenylyl cyclase III

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Introduction

Cilia are microtubule based hair-like structures that protrude from cell surfaces. In general, cilia are classified as motile or non-motile primary cilia. It is considered that motile cilia function mainly as motor organelles, while non-motile cilia are for sensory function, but this classification is not mutually exclusive (1, 2). Primary cilia are found on almost all non-proliferating mammalian cell types (3, 4). Cilia are also found on central neurons, but at first they did not receive much attention as they were thought of as a rudimentary organ (5, 6). However, after the discovery of many receptors and signaling proteins in neuronal cilia, neuronal primary cilia are thought of as cellular antennae sensing the extracellular environment (7-10).

In the retina, which is embryologically considered as a part of the central nervous system, the outer segment (OS) of photoreceptors itself is a highly specialized form of primary cilium, which mediates phototransduction that converts light into an electrical signal (11, 12). Although it shares the common features of cilia, microtubule based axoneme and intraflagellar transport (IFT), it represents a highly specialized form different from the usual hair-like shape. Research on retinal cilia other than the photoreceptor OS is scarce. Allen found isolated cilia (using electron microscopy) in bipolar cells and ganglion cells of guinea pig and human retina and retinal pigment epithelial cells of the human eye (13). Boycott and Hopkins found primary cilia (using Richardson's reduced silver method, light and electron microscopy) in photoreceptors, amacrine, interplexiform, displaced amacrine and ganglion cells of cat and

rabbit retina (14). However, no further research followed on these retinal neuronal cilia, especially using immunohistochemistry or immunofluorescence to discover cilia associated proteins, approaching on their functional aspect.

In this study, we performed immunofluorescence using three different ciliary markers: Arl13b, a small GTPase that is localized on the cilia membrane (15-17); Acetylated α-tubulin, that labels tubulin based axoneme (18); and Adenylyl cyclase III, which converts ATP to cyclic AMP and is known to be enriched in neuronal cilia (10, 19). Through this immunofluorescence, we aimed to determine the expression of primary cilia in the whole retina, and also to characterize the immunohistological antigenicity of primary cilia in the retina.

Materials and methods

Animals

We purchased C57BL/6 mice from Samtako (Osan, Gyeonggi, Korea). All experiments conformed to the statement by the Association for Research in Vision and Ophthalmology regarding the use of animals in research and were approved by Seoul National University Institutional Animal Care and Use Committees (SNU-120111-7). C57BL/6 mice were maintained on a standard 12 hours light-dark cycle with approximately 23°C room temperature, and were fed food and water ad libitum. C57BL/6 mice were sacrificed on postnatal day 26 and eyes were enucleated and fixed with 4% paraformaldehyde in PBS for 24 to 72 hours.

Antibodies

Primary antibody

We used three different markers to detect primary cilia. For the detection of Arl13b, polyclonal rabbit antibody (1:1000) was used. Arl13b antibody was raised in rabbit using recombinant GST fused Arl13b fragments (amino acids 208-428) as similar way described in previous literature (20). Recombinant GST-tagged Arl13b fragments were produced in BL21(DE3) competent cells as recommended by the manufacturer (Stratagene, La Jolla, CA, USA) and purified using the immobilized glutathione agarose resin (Thermo Scientific, Rockford, IL, USA). Purified fusion proteins were used to produce antisera in rabbits (Young In Frontier Co., Seoul, Korea). For the detection of Acetylated

α-tubulin, mouse monoclonal IgG2b antibody (1:1000, Sigma-Aldrich, St. Louis, MO, USA) was used. For the detection of Adenylyl cyclase III, affinity purified goat polyclonal antibody (1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used. Primary antibodies were diluted with dilution buffer; 1% bovine serum albumin, 0.1% cold fish skin gelatin, 0.05% sodium azide and 0.01M phosphate-buffered saline (PBS, pH 7.2).

Secondary antibody

We used Alexa Fluor® 594 donkey anti-rabbit IgG (1:600, Invitrogen, Carlsbad, CA, USA), Alexa Fluor® 488 donkey anti-mouse IgG (1:600, Invitrogen, Carlsbad, CA, USA) and Alexa Fluor® 488 donkey anti-goat IgG (1:600, Invitrogen, Carlsbad, CA, USA) for immunofluorescence. Secondary antibodies were diluted with PBS.

Immunofluorescence

Tissues were embedded in paraffin wax and sectioned at 4μm thickness. Sections were deparaffinized with xylene and rehydrated through graded ethanol; 100%, 100%, 90%, 80%, and 70% ethanol for 5 minutes each. Then, tissues were gently washed in running water for 10 minutes, followed by washing in PBS for another 10 minutes. Antigen retrieval was done with Proteinase K (20μg/ml) at 37°C for 10 minutes followed by 10 minutes at room temperature. After serial washing in PBS for 5 minutes twice, tissue sections were incubated with universal blocking reagent in room temperature for 10 minutes. Tissues were incubated with primary antibodies at 4°C

overnight. After rinsing in PBS for 5 minutes four times, tissues were incubated with secondary antibodies for 2 hours at room temperature in the dark. After an additional PBS rinsing for 5 minutes twice, the sections were mounted on the slide glass with Faramount aqueous mounting medium (Dako, Glostrup, Denmark) and observed under a fluorescence microscope (Nikon Eclipse 80i, Tokyo, Japan). The images were processed with NIS-Elements Microscope Imaging Software ver. 4.00 (Nikon, Japan).

Results

1. Localization of ciliary markers in mouse retina.

Each ciliary marker showed peculiar staining pattern in indirect immunofluorescence. Arl13b was expressed in the photoreceptor layer (PRL), outer plexiform layer (OPL) and weakly in the inner plexiform layer (IPL). In PRL, Arl13b was mostly stained in the inner segment (IS) area, however the staining in connecting cilia (CC) was hardly observable. In OPL, Arl13b showed intense feathery reaction, which was common in all three ciliary marker staining. Arl13b was also weakly and diffusely stained in IPL (Fig. 1A).

Acetylated α-tubulin was expressed in PRL, OPL, IPL and some longitudinal staining patterns were observed throughout the whole retinal thickness including ganglion cell layer (GCL). In PRL, fine speckled staining pattern was observed along the CC area. In OPL, intense feathery pattern staining was observed. One peculiar staining pattern of Acetylated α-tubulin was longitudinal staining, spanning the whole thickness of the retina. Considering its continuity along the whole retinal thickness, it is thought to be the staining of the Müller cells. In the GCL, weak, longitudinal staining continuous from ONL was observed (Fig. 1B).

Adenylyl cyclase III was expressed in the PRL, OPL, GCL and weakly in IPL. In the PRL, Adenylyl cyclase III showed diffuse staining spanning CC and IS with some speckled staining in CC. It also showed strong reaction in

OPL. Different from the other two staining, Adenylyl cyclase III showed diffuse staining in the GCL.

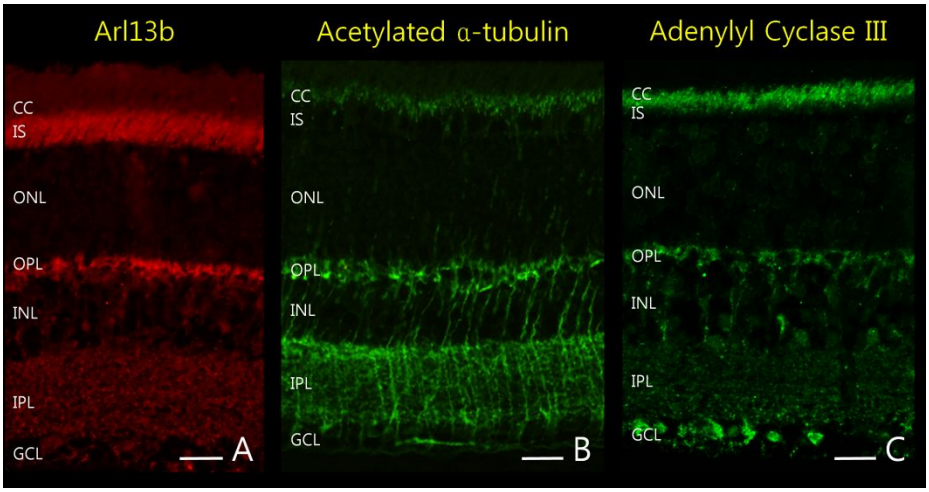

Figure 1. Localization of three different ciliary markers in mouse retina.

Arl13b was expressed in PRL, OPL, and weakly IPL (A). Acetylated α-tubulin was expressed in PRL, OPL, IPL and some longitudinal staining patterns were observed throughout the whole thickness which is thought to be the staining of the Muller cells (B). Adenylyl cyclase III was expressed in PRL, OPL, GCL and weakly in IPL (C). In PRL, Acetylated α-tubulin showed most specific speckled staining pattern in CC area, while Adenylyl cyclase III showed diffuse staining with some speckled staining in CC and Arl13b showed diffuse staining in IS area. All three markers showed strong reaction in OPL.

Abbreviations: PRL, photoreceptor layer; CC, connecting cilium; IS, inner segment of photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars: (A-C) = 20μm.

2. Double staining with anti-Arl13b and anti-Adenylyl cyclase III in mouse retina.

Both Arl13b and Adenylyl cyclase III were expressed in PRL, but the staining pattern was different. Both markers were expressed diffusely in IS area, but Adenylyl cyclase III also showed a speckled pattern near the CC region. Furthermore Adenylyl cyclase III was not expressed in the whole IS layer, but restricted in the outer part of IS (Fig. 2C). In OPL, the region of neuronal synapses, both Arl13b and Adenylyl cyclase III showed strong staining with feathery pattern (Fig. 2A and 2B), and this was colocalized in merged image (Fig. 2C). However, we could not observe any single strand-like shapes that emerge from the cell surfaces that might more specifically suggest the presence of cilia in high power field (x1,000, Fig. 2D). Only Adenylyl cyclase III showed reactivity in GCL (Fig. 2C).

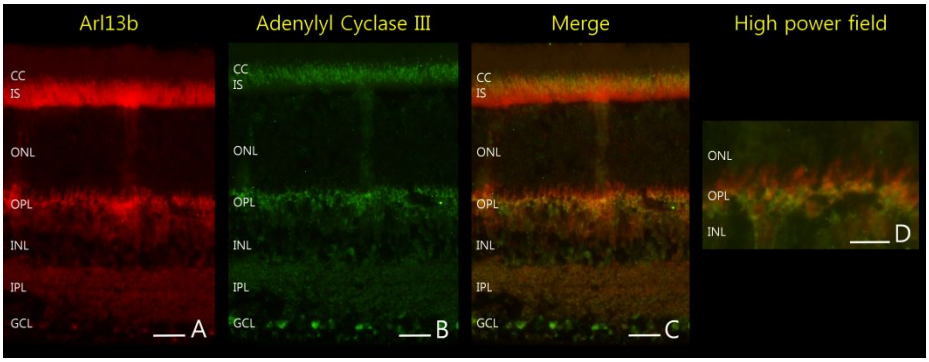

Figure 2. Localization of Arl13b and adenylyl cyclase III in mouse retina. Arl13b was expressed in PRL, OPL and weakly in IPL (A), and adenylyl cyclase III was expressed in PRL, OPL, GCL and weakly in IPL (B). Both showed diffuse staining in PRL. Arl13b was mainly stained in IS area while Adenylyl cyclase III showed some speckled pattern in CC area (A and B). In merged image, Arl13b and Adenylyl cyclase III were colocalized in OPL (C). In high power field, colocalization of both markers was observed but couldn't find any single-strand like feature that emerge from cell surface (D).

Abbreviations: PRL, photoreceptor layer; CC, connecting cilium; IS, inner segment of photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars: (A-C) = 20μm, (D) = 10μm.

3. Double staining with anti-Arl13b and anti-Acetylated α-tubulin in mouse retina.

In the PRL, Arl13b was diffusely stained in IS area while Acetylated α-tubulin showed fine, speckled pattern staining, limited in CC area. This discrimination was prominent in merged image (Fig. 3C). In OPL, both markers showed intense, feathery staining (Fig. 3A and 3B), and this was colocalized in merged image as in double staining with anti-Arl13b and anti-Adenylyl cyclase III (Fig. 3C). However, we could not directly find any single strand-like features protruding from the cell surfaces that might more specifically suggest the presence of cilia in high power field (x1,000, Fig. 2D). Acetylated α-tubulin showed peculiar longitudinal staining spanning the whole thickness of the retina (Fig. 3B). The location, strength and staining pattern of each ciliary marker are summarized in Table 1.

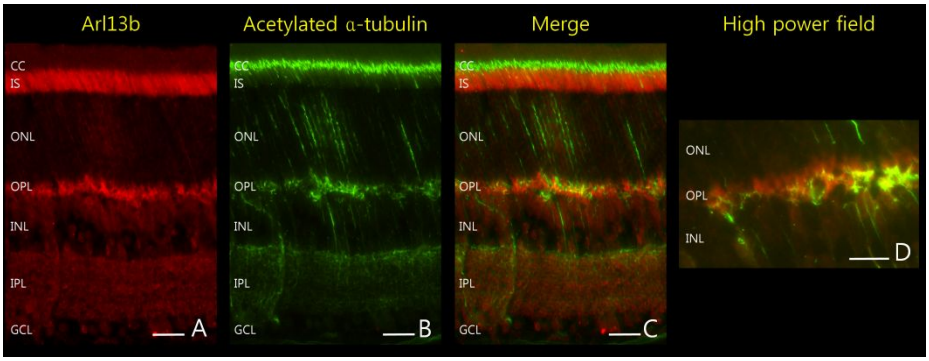

Figure 3. Localization of Arl13b and Acetylated α-tubulin in mouse retina. Arl13b was expressed in PRL, OPL and weakly in IPL (A), and Acetylated α tubulin was expressed in PRL, OPL, IPL and some longitudinal staining patterns were observed throughout the whole thickness, which is thought to be the staining of the Müller cells (B). Acetylated α-tubulin showed specific speckled pattern in CC of PRL (B). In merged image, Arl13b and Acetylated α-tubulin were colocalized in OPL (C). In high power field, colocalization of both markers was observed but could not find any single-strand like feature that emerged from the cell surface (D).

Abbreviations: PRL, photoreceptor layer; CC, connecting cilium; IS, inner segment of photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars: (A-C) = 20μm, (D) = 10μm.

Table 1. A summary table representing immunofluorescence results of three different ciliary markers staining in mouse retina

	PRL	ONL	OPL	INL	IPL	GCL
Arl13b	++	-	++	-	+/-	-
	Diffuse		Feathery		Diffuse	
Acetylated α tubulin	++	+	++	+	++	+/-
	Speckled	Longitudinal	Feathery	Longitudinal	Diffuse and Longitudinal	Longitudinal
Adenylyl Cyclase III	++	-	++	-	+/-	++
	Diffuse		Feathery		Diffuse	Diffuse

Weak labeling is denoted by +/-, moderate labeling by +, strong labeling by ++ and no labeling by -.

Abbreviations: PRL, photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

Discussion

In this study, we performed immunofluorescence using three different ciliary markers to discover the expression of primary cilia and find out their immunohistological features in mouse retina. In PRL, only Acetylated α-tubulin showed fine speckled pattern in photoreceptor CC area, while the other two markers showed diffuse staining in the IS and the CC area. One possible explanation of this difference of staining patterns among different ciliary markers is that we used antibodies with different characteristics. We used polyclonal antibodies for Arl13b and Adenylyl cyclase III, while monoclonal antibody for Acetylated α-tubulin, and this might led to a more specific reaction in Acetylated α-tubulin staining.

Arl13b is a member of the Arf (ADP-ribosylation factor)/Arl (Arf-like)-family small GTPases (15). It is known that Arl13b is localized to the ciliary membrane (16), so we did not treat the tissue with Triton X-100, a detergent that solubilizes membrane-bound proteins, and there is no possibility that cilia are broken down by unrecognized routine procedures in our experiment. Our staining pattern of Arl13b in PRL is similar with previous study, which used rabbit polyclonal antibodies for Arl13b, which was raised similarly with ours. It also showed intense and rather diffuse staining throughout the CC and IS area (21), and this might be the immunohistological features of PRL.

In a previous report on the localization of Adenylyl cyclase proteins in rodent retina (22), Adenylyl cyclase III was localized on OPL, INL, IPL and GCL, but not in PRL. In their experiment, Adenylyl cyclase III was labeled in

PRL outer segments, however, this labeling was observed after absorption by pre-immune peptide, and they concluded that labeling in PRL is non-specific. We think that our diffuse staining in PRL by Adenylyl cyclase III also has the possibility of non-specific staining.

In PRL, we only demonstrated axonemes of CC by staining of Acetylated α-tubulin, however we observed diffuse and possibly non-specific reaction with Arl13b and Adenylyl cyclase III. It is suggested that the PRL CC, the specialized form of cilia, has different immunohistologic and functional features with cilia found in other cells. Adenylyl cyclase III is known for abundant in neuronal cilia (9, 23), and it is also known to be associated with ciliary length control (24). It seems that although PRL CC shares many features with primary cilia, it lacks the active mechanism that sensing and interacting with extracellular environment like cilia in other cells, especially the neuronal cilia.

The OPL was the site where all three ciliary markers showed strong reactivity. Compared to previous literatures, which investigated retinal ciliated cells, the possible retinal cells are bipolar cells and interplexiform cells. Allen reported finding retinal cilia in bipolar cells, which were more commonly found in neurons along the outer and inner edges of INL (13). Boycott and Hopkins found cilia in interplexiform cells (14). However, we could not find any single strand-like pattern, protruding directly from the cell surface that might more specifically suggest the presence of cilia. It might be related with the extent of magnification we used in this study. Allen reported that the

length of retinal neuronal cilia varied from approximately 1.2 to 1.8 μm using electron microscopy (13). We used up to x1,000 magnification for high power observations, but this might have been insufficient to resolve retinal cilia. Furthermore, it is known that the ciliary length is modulated according to its environmental changes (25). Therefore, we might need much higher resolution (e.g. electron microscopy) to assure the observation of primary cilia in retina.

Our staining pattern in OPL corresponds to the location and direction of processes of horizontal cells. If we suppose that the role of primary cilia in retina is to sense the changes in the extracellular environment, the cilia should be placed near vessels, because retina is protected from the extracellular environment by the 'blood-retinal barrier'. In that sense, the OPL, where the outer deeper plexus exists, could be a candidate site for primary cilia. Also, there are reports that the horizontal cell processes are in contact with retinal capillaries (26, 27). Although Boycott and Hopkins reported horizontal cell as non-ciliated cells, as they pointed out, there are limitations in terms of resolution and staining methods (14). Thus, considering our strong horizontal immunoreaction in OPL, common in all three ciliary markers, might suggest the possibility that horizontal cells possess cilia and interact with the extracellular environment.

In a study of the distribution of IFT molecules in the retina, IFT20, -52, and -57 were localized in the postsynaptic dendritic terminals and dendritic shafts of secondary retinal neurons in the OPL. These IFT proteins were associated

with vesicle-like structures and the authors reported that the IFT system is also participating in the vesicular transport at postsynaptic dendritic terminals (28). There is also increasing evidence that IFT proteins are not only involved in the ciliary assembly or disassembly process, but are involved in the exocytosis process (29-31) It is interesting that not only the IFT proteins but also the other ciliary markers were observed in OPL as in our study. Our staining result in OPL was intense and showed a feathery pattern along the entire neuronal synapses. One possible interpretation of this is that dendritic process of secondary neurons itself shares ciliary characteristics. Recent studies suggested the role of primary cilia as a secretory organelle, or striking similarities between immunological synapses and cilia (31, 32). Thus, there is a possibility that the neuronal synapses in OPL shares ciliary characteristics.

In addition to PRL and OPL staining, Acetylated α-tubulin also showed peculiar longitudinal staining spanning the whole thickness of the retina. Although we did not perform double staining the tissue with Müller cell markers (such as anti-Vimentin), considering its continuity from ONL to GCL and its similarity with staining pattern of Müller cells as described in previous literature (33), is thought to be staining the Müller cells.

In GCL, where the retinal neurons that are directly connected with brain exist, only Adenylyl cyclase III showed strong reactivity, while Arl13b showed negative result and Acetylated α-tubulin showed weak longitudinal staining that is thought to be of Müller cells. Considering that the marker for cilia on most cell types, Acetylated tubulin, is not specific for neuronal cilia

and labels neuronal processes, and the majority of neuronal cilia are positive for Adenylyl cyclase III (9, 23), it seems plausible that ganglion cell, which is in close relation with central nervous system, shows reactivity to Adenylyl cyclase III. In our results, it is also clear that the Acetylated α-tubulin does not resolve the ciliary axoneme against the cytoplasmic tubuli fluorescence. As in PRL CC, where adjacent cytoplasmic tubuli fluorescence is absent, we could easily detect axonemes, however in other retinal layers, it was difficult to resolve axoneme against the tubuli fluorescence from neuronal processes. However we could not find any strand-like shape protruding from the cell surface either but showed diffuse staining in GCL, limiting the interpretation of it.

In summary, in immunofluorescence with three different ciliary markers in mouse retina, we found positive reaction in the PRL, OPL and GCL. We could not directly prove the strand-like shape of cilia in those areas, but our results suggest the possibility of the presence of primary cilia in the OPL and GCL in addition to the PRL OS. Especially in the OPL, the intense staining of all three markers in a feathery pattern along the neuronal synapses suggests that neuronal processes might share the features of cilia. Further study using electron microscopy combined to immunofluorescence assay is needed to resolve the fine structures of immunoreactive site suggested in this study.

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국 문 초 록

서론: 최근 원발섬모 (Primary cilia)는 세포 주변 환경을 인지하는 감각기관으로 주목 받고 있으며, 대부분의 포유류의 세포에서 하나의 원발섬모를 가지고 있는 것이 알려져 있다. 망막에서는 시세포 외절이 특수한 형태로 분화한 원발섬모라는 것이 잘 알려져 있다. 하지만 시세포 이외의 망막부위에서의 원발섬모에 대한 연구는 거의 이루어지지 않고 있다. 이에 이번 연구에서는 세 가지 섬모 표지자를 이용하여 생쥐 망막에서의 원발섬모 발현 여부를 알아보고자 하였다.

방법: C57BL/6 생쥐의 안구를 출생 후 26일 째 적출하였다. 4㎛ 두께 파라핀 절편을 만든 후 면역형광염색법을 이용하여 세 가지 섬모 표지자인 Arl13b, Acetylated α tubulin, Adenylyl cyclase III 의 발현 양상을 살펴보았다.

결과: Arl13b는 시세포층, 외망상층에서 염색되는 것이 관찰되었고, 내망상층에서는 약한 염색이 관찰되었다. Adenylyl cyclase III는 Arl13b와 유사한 염색 양상을 보였으며, 추가적으로 시신경절 세포층에서

도 염색이 관찰되었다. Acetylated α tubulin은 시세포층, 외망상층, 내망상층에서 염색이 관찰되었으며, 망막전층을 통과하는 세로방향의 염색이 관찰되었는데, 이는 뮬러세포가 염색된 것으로 생각된다. 시세포층에서 Arl13b와 Adenylyl cyclase III는 시세포 내절부위에서 미만성의 염색양상을 보인 반면, Acetylated α tubulin은 시세포 연결섬모 (connecting cilia) 부위에서 점상의 염색양상을 보였다. 외망상층에서는 세 가지 표지자 모두 시신경 연접부위에서 강한 깃털 모양의 염색소견을 보여주었다. 하지만 세포 하나하나에서 직접적으로 돌출되는 실모양의 염색 소견은 관찰할 수 없었다.

결론: 세 가지 섬모 표지자를 이용한 면역형광염색을 해 본 결과, 망막의 시세포층, 외망상층, 시신경절 세포층에서 염색이 된 것을 발견하였다. 비록 이번 실험에서 직접적인 실모양의 섬모를 관찰할 수는 없었지만, 이번 연구결과는 망막의 시세포층 외에 외망상층, 시신경절 세포층에서도 역시 원발섬모가 존재할 가능성이 있음을 시사한다. 특히 세 가지 섬모 표지자에 모두 양성 반응을 보인 외망상층의 신경 연접부위를 보면, 신경돌기 자체가 섬모의 특성을 공유할 수 있음을 시사한다.

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주요어: 원발섬모; 생쥐 망막; Arl13b; Acetylated α tubulin; Adenylyl cyclase III

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