Graduate School of Biological Sciences Doctoral Thesis Abstract

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Title	Analysis of primary cilia formation and function in neurotoxic A1 astrocytes		

The primary cilia are antenna-like and immotile projection extending as a solitary unit from the basal body, derived from the centrosomal mother centriole. This tiny organelle can be found on the surface of most types of mammalian cells and plays a crucial role in coordinating centers for signal transduction to regulate several biological events, such as cell cycle progression. Astrocytes are abundant glial cells that play a regulatory role in maintaining homeostasis in the central nervous system (CNS). Although the importance of astrocytes in homeostasis, accumulated evidence has revealed that astrocytes also have a significant role in neuroinflammation. There are two types of reactive astrocytes; neurotoxic A1 and neuroprotective A2. A1 astrocytes are strongly induced by CNS injury and acute insults like stroke, whereas A2 astrocytes are induced by ischemia. In addition, several studies recently reported that A1 astrocytes were also found in chronic neurodegenerative diseases such as Alzheimer's, Parkinson's, and multiple sclerosis. However, the physiological function of the primary cilia, especially the physiological relevance between A1 astrocytes and primary cilia, is poorly understood.

To investigate primary cilia formation in A1 astrocytes, cortical glial mixture cells derived from postnatal-7 (P7) mice were treated with LPS for 24h, 48h, and 72h and immunostained with primary cilia marker (ARL13B). I co-stained the cells with an astrocytes marker (GFAP) and an A1 astrocytes marker (C3). In this experiment, I revealed that GFAP+C3+ A1 astrocytes were significantly increased following LPS stimulation compared to the control. In this condition, the length of astrocytic cilia was significantly elongated than the control. Similarly, primary cilia length was elongated in purified astrocytes culture after 24 h exposure to IL-1α, TNF-α, and

C1q. These data suggest that A1 astrocytic cilia were elongated compared to non-reactive astrocytes.

To elucidate whether A1 astrocytes differentiation or primary cilia elongation comes first, I stimulated purified astrocytes with earlier time points than 24 hours, which were 0 h, 1 h, 3 h, 6 h, and 16 h. The time points dependency experiment suggested that exposure of IL-1α, TNF-α, and C1q for 6 hours was sufficient to induce A1 astrocytes. In contrast, when I measured the primary cilia length, I found that the primary cilia were significantly longer after 1-hour stimulation. Therefore, these data suggested that C3 expression is induced following the elongation of the primary cilia.

Having shown that primary cilia elongation is associated with A1 astrocytes differentiation, I next conducted gene silencing of IFT88 to disassemble primary cilia. I discovered a reduction of IFT88 expression and primary cilia formation after siRNA treatment, as indicated by Western blot analysis and immunostaining. Notably, C3 expression in immunostaining data was significantly lessened when cells were transfected with siRNA targeting IFT88, suggesting that primary cilia might play a role in A1 astrocytes differentiation.

To further examine the function of primary cilia in mice, *in vivo* study was committed. As some papers reported that LPS injection in mice significantly increased A1 astrocytes, I researched whether astrocytic primary cilia are elongated *in vivo*. To visualize the primary cilia, I immunostained brain tissues and saw the tendency for the primary cilia to be extended in LPS-injected mice compared to PBS-injected mice. This data suggested that induction of LPS to mice could elongate astrocytic primary cilia *in vivo*. Next, I gauged mouse recognition by performing the novel object recognition test (NOR). LPS administration in mice showed a significantly diminished preference for the novel object. In an open field test (OFT), locomotor activity measurement did not display hyperactivity in LPS-injected mice, although I found that in 2-year-old mice. These data suggested that neuroinflammation caused by LPS induction caused recognition impairment. To confirm the physiological function of the primary cilia in neuroinflammatory astrocytes and mouse behavior, we generated cKO mice lacking IFT88 in astrocytes. Based on this experiment, we found that LPS affect was disappeared in cKO mice.

Signal transduction via heterotrimeric G proteins is fundamental for mediating a broad range of cellular responses in the primary cilia. As my data suggested that primary cilia may restrain A1 astrocytes differentiation, I hypothesized if the signaling pathway through GPCR in primary cilia regulated A1 astrocytes differentiation. I searched astrocytes transcriptome changes to inflammation from literature and focused on an orphan G protein-coupled receptor (GPCR) known as GPR179, which is significantly upregulated 24 hours after LPS injection to mice. It has been reported that GPR179 is highly localized in human retinal photoreceptor cells, which have a typical structure resembling primary cilia. To explore GPR179 localization in the primary cilia, I used NIH3T3 cells, which endogenously express GPR179. To study the function of GPR179, we generated GPR179 KO NIH3T3 cells. I found that primary cilia length was shorter in GPR179 KO cells compared to WT NIH3T3 cells. Even though this experiment was performed for the first time (n=1), the results suggested that GPR179 tends to regulate primary cilia length and function.

Altogether, the current findings suggest that primary cilia in astrocytes regulates A1 astrocytes differentiation, and GPR179 is one of the candidates controlling primary cilia formation and astrocyte function. In the future, several extensive studies must be executed to confirm the role of the primary cilia in A1 astrocytes.