

SPOTLIGHT

Cilia bent out of shape over dysfunctional astrocyte mitochondria

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Mitochondrial dysfunction in astrocytes drives neurodegenerative brain pathology. In this issue, Ignatenko et al. (2022. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202203019>) discover a novel connection between cilia and mitochondria in astrocytes, whereby mitochondrial dysfunction leads to abnormal cilia structure and a motile cilia program.

Astrocytes are the most abundant glial cell in the brain and serve many critical functions in the central nervous system (CNS) including regulating synapses, the blood-brain barrier, and metabolism. In response to insults in the brain such as stress, injury, or disease, astrocytes transition from a homeostatic role to a reactive state. Reactive astrocytes display changes in morphology, function, and molecular programs to adapt to the insult. This reactive state serves as immediate neuroprotection, but prolonged reactivity can have toxic effects (1). Astrocytes are emerging as key players in understanding the pathology of neurological diseases. However, the molecular consequences of metabolic stress in neurological diseases remain poorly understood.

Many neurological diseases present with primary or secondary mitochondrial dysfunction. Impaired mitochondrial function activates the integrated stress response, a pathway that communicates between organelles to restore metabolic homeostasis (2). Ignatenko and colleagues previously developed a mouse model of mitochondrial dysfunction by deleting a mitochondrial DNA-helicase, *Twinkle* (*Twnk*), resulting in loss of mitochondrial DNA (3). They used this model to explore differences between astrocytes and neurons in their response to mitochondrial dysfunction. Loss of *Twnk* specifically in astrocytes (*TwkKO^{astro}*) causes neurodegenerative brain pathology and

induces the mitochondrial integrated stress response (ISR^{mt}). Importantly, loss of *Twnk* in neurons resulted in acute and delayed neurodegeneration with no ISR^{mt}. This work indicates that the response to mitochondrial dysfunction in the brain is cell-type specific and highlights the need to study mechanisms of mitochondrial stress in astrocytes.

In this issue, Ignatenko et al. (4) examine the molecular changes in astrocytes with mitochondrial dysfunction to better understand mechanisms of metabolic stress in neurodegenerative diseases (4). The authors used magnetic sorting to purify astrocytes from the *TwkKO^{astro}* mice and performed bulk RNA sequencing. They found upregulation of genes involved in ISR^{mt} and reactive astrocytes, indicating that astrocyte mitochondrial dysfunction causes metabolic stress and astrocyte reactivity. By following up in vivo, they show that *TwkKO^{astro}* astrocytes exhibit disrupted lipid storage and increased GFAP staining, which are indications of impaired metabolism and astrocyte reactivity, respectively.

Interestingly, Ignatenko et al. (4) found the top upregulated pathways revealed genes linked to cilia. Cilia are specialized microtubule-based organelles that project from the cell membrane. Cilia are present on nearly all cell types with critical functions in sensing and responding to stimuli. There are two types of cilia, primary and motile, that have distinct functions and structural

components. Primary cilia are singular, stationary, and serve as signaling centers, whereas motile cilia are often present in multiples and move to generate fluid flow. All cilia have a central axoneme comprised of doublet microtubules. Motile cilia are distinguished by the central pair of microtubules along with additional components that produce force to generate motion. The authors found that the genes induced in *TwkKO^{astro}* are all components specific to motile cilia. Astrocytes possess one primary cilium, so it is very surprising that genes unique to motile cilia are induced in *TwkKO^{astro}* astrocytes (5).

To explain the adoption of a motile cilia program, Ignatenko and colleagues identified that binding sites of the RFX transcription factor family, known to regulate ciliogenesis, are over-represented in genes upregulated in *TwkKO^{astro}*. Furthermore, *Foxj1*, a transcription factor and regulator of motile ciliogenesis, is significantly upregulated in *TwkKO^{astro}* astrocytes. *Foxj1* is abundantly expressed in multi-ciliated ependymal cells that line brain ventricles and help move cerebral spinal fluid. Remarkably, an increase in *Foxj1* expression is observed in the cortex of *TwkKO^{astro}*. One limitation is that the GFAP-73.12-Cre driver used to target astrocytes is also active in ependymal cells. The authors examined the motile cilia on ependymal cells and observed normal morphology. This suggests that

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astrocyte cilia respond differently than other CNS cells to mitochondrial stress. This is supported by work in mouse models with heart-specific deletion of *Twink*, which show no changes in motile cilia genes. Together, these indicate that mitochondrial dysfunction has cell-type specific molecular consequences.

The authors explored this motile cilia transcriptional program further by imaging ARL13B, a protein enriched along the ciliary membrane, in astrocyte cilia. *TwkKO^{astro}* astrocytes maintain a singular cilium, though there are striking changes to cilia including elongation and distorted morphology. This aligns with previous work showing changes in astrocyte cilia morphology in response to CNS insults and in neurodegenerative diseases (6, 7). However, this approach does not demonstrate whether there are changes to the structure of the ciliary axoneme. Future studies using advanced imaging techniques should enable a more detailed analysis of the changes in cilia structure. Next, they stained for motile cilia proteins encoded by the upregulated transcripts. These proteins did not localize to astrocyte cilia; and instead, one showed cytoplasmic expression. This raises the possibility that mitochondrial dysfunction disrupts trafficking of motile cilia components.

This paper reveals an unappreciated connection in astrocytes between mitochondria and cilia by illustrating that mitochondrial stress causes dramatic changes in the ciliary transcriptional program and in vivo cilia structure. Cilia research in astrocytes trails behind other cell types in the CNS, leaving our knowledge of astrocyte cilia deficient. These results prompt an important discussion about the cellular heterogeneity of cilia and presentation of motile and primary cilia. It is critical to understand the structure of astrocyte cilia in order to interpret changes that occur in disease states. As signaling hubs, changes to primary cilia structure could disrupt the signaling pathways linked to cilia. An important question for the authors to address next: What is the functional significance of altered cilia length in astrocytes?

Cilia are frequently studied in isolation, and it is important to understand how they function with other organelles. This work suggests that a signaling axis exists from mitochondria to cilia in astrocytes, and perturbations to this communication have implications for neurological diseases. This raises several intriguing questions including what are the signaling pathways that underlie mitochondria-cilia communication?

Is the communication one-way or two-way? If cilia structure is perturbed in astrocytes, are there consequences to mitochondrial function? Finally, do signaling networks exist between other organelles and cilia in astrocytes? This publication from Ignatenko et al. (4) raises new questions and possibilities for future work studying astrocyte primary cilia and how they can inform our knowledge of neurological disease processes.

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