

Zfp423 regulates Sonic hedgehog signaling via primary cilium function

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April 17, 2023

Abstract

Available from: HONG, C.-J., & Hamilton, B. A. (2016). Zfp423 regulates Sonic hedgehog signaling via primary cilium function. doi:10.1101/046961



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DATE RECEIVED:

September 18, 2016

DOI:

10.15200/winn.147699.98895

ARCHIVED:

October 20, 2016

KEYWORDS:

Primary Cilia, ASP2016Fall

CITATION:

Bailey Allard, Wei Wang, Zfp423 regulates Sonic hedgehog signaling via primary cilium function, *The Winnower* 3:e147699.98895, 2016, DOI: 10.15200/winn.147699.98895

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The manuscript titled “Zfp423 regulates Sonic hedgehog signaling via primary cilium function” seeks to explain the role of the nuclear transcription factor, *Zfp423*, in ciliopathy related clinical manifestations of the brain. The authors use a multi-faceted approach to investigate the role of *Zfp423*. They show that *Zfp423* localizes to granule cell precursors, and that loss of *Zfp423* leads to a decrease in proliferation, primary cilia structure abnormalities, and decreases the localization of *Smoothened* to the primary cilia. The authors also investigate the nuclear binding of *Zfp423* to ciliary genes to determine a link between *Zfp423* and the primary cilia. Through these studies, they identify *Tulp3* as a potential functional target of *Zfp423* which, once expression levels are lowered, can in part rescue the *Smoothened* translocation defects of *Zfp423* loss. In conclusion, the authors suggest that *Zfp423* deficiency is a ciliopathy.

The diverse range of experiments and the novelty of a nuclear transcription factor affecting the structure and signaling of the primary cilium lead this paper in an exciting direction. However, to aid in the interpretation of the data, further evidence would help support the following claims:

- 1) Zfp423 is expressed in cerebellar granule cell precursors – In figure 1, the authors show evidence of specific staining for Zfp423. To better interpret the data, it would help to co-localize this staining with known markers of the different regions (particularly the cerebellar granule cell precursor region) to improve our understanding of the spatial distribution of Zfp423 within these regions.
- 2) Zfp423-deficient precursors have abnormal cilium morphology – In figures 3 and 4, the authors conclude that loss of Zfp423 causes cilia abnormalities including changes in cilia volume, basal body volume, cilia length, and base width of the primary cilia. However, the representative images and graphs do not clearly display this information. Perhaps scanning electron microscopy images of the primary cilia would clarify the cilia abnormalities resulting from loss of Zfp423 and would allow both the authors and the readers to better appreciate the differences, especially in cilia volume and base width. Perhaps it would also be beneficial to label the membrane surrounding the primary cilia to better understand the increase in base width of the primary cilia.
- 3) ZNF423 knockdown quantitatively alters *Smoothened* translocation – In figure 5, the authors conclude that the proportion of cilia with high levels of SmoGFP is decreased while the proportion of cilia with low levels of SmoGFP is increased. Although this data implies that there are likely abnormalities in Hedgehog signaling, it would further strengthen the argument for the importance of

Zfp423 in this cilia based signaling pathway if the RNA or protein levels of Hedgehog target genes were also altered, indicating a downstream effect of the *Smoothened* translocation defects.

4) ZNF423 deficiency reduces IFT88 translocation into cilia – In figure 6, the authors show images, both *in-vitro* and *in-vivo*, which support the idea that loss of ZNF423/Zfp423 leads to a decrease in IFT88 levels within the primary cilia. Although the images are clear, it is known that IFT88 is necessary for cilia function and that loss of IFT88 leads to a decrease in cilia length. However, the ciliary images presented in figures 3 and 4 do not show a decrease in cilia length, but possibly even a increase in cilia length. If the authors make these two claims (increase in cilia length and decrease in IFT88 in the primary cilia), data or speculation supporting a possible mechanism for this unexpected result would help the reader further understand and interpret the data.

Throughout the paper, the authors show representative images to accompany the quantification of their data. To better understand the data, it would be helpful to ensure that images clearly represent the data obtained. In particular, the following figures and images may need to be revisited:

- 1) Figure 2 – The authors show an image of the Zfp423 kd cells where the GFP expression and the BrdU incorporation are mutually exclusive. However, the mitotic index shown indicates that the two may not always be exclusive of one another. Perhaps, it would be nice for the reader to see images representing one standard deviation away from the average.
- 2) Figure 5 – The authors state that with ZNF423 KD, there is an increase (110%) in acetylated α tubulin and a decrease (95%) in IFT88 expression. Since these differences are likely too small to be seen by the eye, it would be helpful to know if these percentages were taken from a single blot or whether they are reproducible, which would help the reader understand whether the 5-10% difference seen is or is not within the margin of error.
- 3) Figure 9 – The authors state that with the shRNA, the Tulp3 levels are reduced 70-90% of the control levels. However, the image presented does not seem to show these differences compared to the control lane.

After reading this article, we were left with a few questions that might be able to be answered with experiments that have already been performed, or might be of interest for future publications.

- 1) Can you speculate on potential functional consequences of increased base width of the primary cilia?
- 2) The first cilia gene identified in the RNA seq has a rank value of 31. What was the gene that was most significantly misregulated?
- 3) Gli1 was identified as a misregulated gene in the RNA seq data, but had a rank value of 1016. Do you think that the translocation of *Smoothened* to the primary cilia is a primary cause of the defects when Hedgehog target genes are not some of the most prominently misregulated genes?
- 4) Do you hypothesize that other pathways may be contributing to the abnormalities seen with loss of Zfp423?
- 5) After decreasing levels of TULP3 in figure 9, are you able to rescue other respects of *Znf423* loss, including IFT88 localization or primary cilia structure?