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The correlative hypotheses between Pitchfork and Kif3a in palate development☆

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ABSTRACT

It is well known that dysfunction of primary cilia during embryonic development causes a range of developmental disorders such as cleft lip and palate, lung, kidney and heart disease. Both Pitchfork and Kinesin family member 3a (Kif3a) are associating with primary cilia, but whether there is a correlation between them are still inconclusive. Our research confirmed that Pitchfork over-expression induced lateral cleft palate and primary cilia disassembly during palate development. We also demonstrated that Sonic hedgehog (Shh) and Patched1 (Ptc1) expression levels were altering in the over-expressed Pitchfork group during palate development. Then we observed by consulting a vast amount of literature that specific knockout of the Kif3a also induced lateral cleft palate and expended the expression domains of Shh and Gli1 during palate development. Furthermore, loss of the Kif3a results in disassembly of the primary cilia and eventually leads to abnormal palatal development. Finally, we found that both Pitchfork and Kif3a are accumulating at the basal body and ciliary necklace during the early phase of cilia assembly and disassembly and both of them are involved in ciliary transport. Based on the above evidence, we hypotheses that there may be a potential correlation between Pitchfork and Kif3a, that could regulate primary cilia disassembly during palate development.

Introduction

A variety of developmental disorders which caused by a human congenital gene mutation that leads to structural and functional defects of the primary cilia are collectively called Ciliopathies [1]. It is well known that many Ciliopathies, including cleft lip and palate, are associated with abnormalities of primary cilia and Shh signaling pathway, but the occurrence mechanisms are largely unknown. To gain insight into the mechanisms that produce cleft palate, we opted for an approach that, rather than disrupting any component of a signaling pathway such as Shh, disrupted the ability of the cell to respond to signals in their environment. Thus, we utilized a ubiquitous organelle, the primary cilia. Primary cilia have been reported to be required for Hedgehog signal transduction [2].

Pitchfork is a mouse embryonic node gene, discovered in 2010. It plays an important role during mouse palate development by regulating primary cilia dis-assembly. We over-expressed Pitchfork by constructing a lentiviral transfection plasmid and infecting in an E13.5 Institute of Cancer Research (ICR) mice at a previous study to observing the condition of the primary cilia during the mouse palate development [3].

The formation of primary cilia begins from the basal body, however, it lacks a protein synthetic system for the assembly, maintenance, and disassembly. So it is necessary to transport the required substances from intracellular through the Intraflagellar transport (IFT) [4]. As an anterograde transport kinesin, Kif3a plays an important role in the proteins transportation in the cilia [5]. Loss of the IFT protein Kif3a results in non-functional primary cilia [6], and most studies suggest that a mutation in Kif3a reduces Hedgehog signal transduction [7]. Although the relationship between Pitchfork and Kif3a are still inconclusive, it is certain that they are inextricably linked to primary cilia. Therefore, researching the correlation between Pitchfork and Kif3a during the mouse palate development may provide a new idea to overcome the Ciliopathies.

The hypotheses

When it comes to the current research about Ciliopathies, Pitchfork

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and Kif3a, have to be mentioned. We did an amount of research and literature review found that there is an inseparable relationship between them. So we hypothesized that there may be a potential correlation between Pitchfork and Kif3a, that could regulate primary cilia disassembly during palate development. Any mutation occurs in either of them may cause abnormal expression of downstream genes and morphological changes eventually.

Evaluation of the hypotheses

Pitchfork

Pitchfork is a mouse embryonic node gene that first reported in 2010. According to the paper [8], they did a High-resolution colocalization study at the mouse node which is an ideal in vivo model for the study of cilia assembly and disassembly. The result shows that Pitchfork is localized apical to the adherens junction at the base of the primary cilium in a ciliary necklace-like pattern and colocalizes with centrosomal/basal body proteins Kif3a, Cep135, and phAurA during the early steps of cilia assembly and disassembly, when structural, functional, and regulatory proteins are delivered to cilia. They also found that the motor protein Kif3a physically interact with Pitchfork in HEK293T cells. It shows that Pitchfork is involved in MT-dependent vesicular trafficking and physically interact with vesicular targeting complexes, suggesting a function in ciliary transport. Bomi Jung et al. also found that Pitchfork and the seven-transmembrane receptor Smoothened (Smo) is bridged by a G protein-coupled receptor-associated sorting protein (Gprasp2) into heterotrimeric primary cilia targeted complex, which transports Smo to primary cilia [10]. Another study shows haploinsufficiency for Pitchfork leads to a unique cilia duplication phenotype at the mouse node, which usually shows absent, small, or bulged cilia [9]. We constructed the lentivirus transfer plasmid pCDH-Pitchfork and infecting in the E13.5 ICR mice to overexpressing the Pitchfork. Palatal shelves were isolated from E13.5 ICR mice (purchased from Koatech, Pyeongtaek, Korea) maxilla and cultured in medium without fetal bovine serum at 37 °C and under 5% CO2 for 48 h. Scanning electron microscopy confirmed that 25 specimens were observed under low magnification, and lateral cleft palate was found in all of the Pitchfork overexpression group and the primary cilia reduced compared with the control group (Fig. 1). What's more, we also confirmed that Pitchfork over-expression resulted in up-regulated Shh and Ptc1 expression and led to an up-regulation of the Shh signaling pathway activity [3]. Bomi Jung et al. also confirmed that the expression of Gli1, Gli2 protein, and mRNA induced by Shh is dependent on the concentration of Pitchfork, and it is found that Pitchfork may be an enhancer of Shh signal by kinetic model analysis [10]. Their views are consistent with our research findings.

In summary, Pitchfork colocalized with Kif3a and involved in ciliary transport. Over-expressing the Pitchfork could up-regulate the Shh signaling pathway, reducing the primary cilia and lead to cleft palate. Interestingly, we found that Kif3a also plays an important role in primary cilia.

Kif3a

Kif3a is a sub-unit of heterotrimeric kinesin2, belonging to the N-kinesin, a microtubule (MT) positive-directed motor protein. Heterotrimeric kinesin2 consists of two sub-units (Kif3a and Kif3b), and the carrier protein KAP3 [11]. As mentioned before, primary cilia cannot synthesize the proteins required for its assembly, maintenance, and dis-assembly, so it has to rely on the IFT [12]. Studies have shown that lack of Kif3a will fail to assembly and maintenance of primary cilia [13]. IFT protein Kif3a mutant shared a number of features found in human conditions including cleft palate. Samantha et al. found that in Kif3a specific knockout embryos, cranial neural crest cells do not extend primary cilia, and the bones of the palate and ventral cranial midline, including the maxilla, trabecular basal plate, Palatine and basisphenoid were either laterally displaced, or if they were mid-line elements, reduced to bony nodules or absent [14]. In this paper, they also confirmed that elimination of the IFT protein Kif3a leads to excessive Hedgehog responsiveness in facial mesenchyme, which is accompanied by broader expression domains of Gli1, Ptc1, and Shh, and broader domains of Gli1 expression correspond to areas of enhanced neural crest cell proliferation in the facial prominences of Kif3a conditional knockouts [14]. Taken together, the absence of the Kif3a will fail to maintain the primary cilia. It also up-regulated the Hedgehog signaling pathway, and lead to palate cleft eventually.

Discussion

Before 1950, primary cilia were considered to be the degenerative organelles of primitive cells, but in recent years, it has become a research hot-spot in the biomedical field [13]. Primary cilia is a kind of signal receptor that widely existed on the surface of mammalian cells. It can perceive the physical and chemical stimulation from the extracellular and transmit these signals to the cells, then causing the cell response [14]. Many receptors and ion channels are distributed on the primary ciliary membrane, however, the relationship with the Hedgehog signaling pathway is particularly close [15]. The Hedgehog is segment-polarity genes that found in fruit flies in 1980 [16]. The secretory signal proteins which encoded by Hedgehog, play an important role in the formation of embryonic developmental organs. In mammalian cells, Ptc1, Smo and its downstream transcription factors Gli1, Gli2 and Gli3 which are the core elements of the Hh signaling pathway, are distributed on the primary cilia [17]. Therefore, when the structure or function of primary cilia broke down, Hh signaling pathway will be out of control, afterward leading to a series of developmental abnormalities, including cleft lip and palate, Bardet-Bird syndrome (BBS), Meckel-Gruber syndrome (MKS), Ellis-van Creveld syndrome (EVC) [18].

In this paper, we propose hypotheses that there may be a potential correlation between Pitchfork and Kif3a, which could regulate primary cilia dis-assembly during palate development. The following arguments support our hypotheses:

(1) Whether Pitchfork over-expressed or specific knockout, the number of primary cilia will significantly be reduced. In Kif3a specific knockout embryos, the number of primary cilia also reduced remarkably.
(2) Over-expressing Pitchfork up-regulated Shh signaling pathway. Loss of Kif3a results in an expansion of the Hedgehog signaling pathway and the expression of Gli1, Ptc1, Shh.
(3) After over-expressing Pitchfork, the lateral cleft palate was observed in E13.5 ICR mice Palatal shelves. Palate development also is hampered in Kif3a specific knockout embryos, and manifest as the bones of the palate were either laterally displaced or absent.
(4) Pitchfork and Kif3a co-localizes in the basal body and ciliary necklace during the early phase of cilia assembly and dis-assembly. And they both involved in ciliary transport.

At present, Ciliopathies has been widely studied around the world which is caused by structural or functional defects in primary cilia, but the specific regulatory mechanism during palate development is still unclear. Based on the results of previous studies and literature review, we raised speculation about the potential relationship between Pitchfork and Kif3a which is related to primary cilia dis-assembly during mouse palate development. We believe that our hypotheses should be urgently needed by the biomedical field, because it may bring a different research approach about Ciliopathies in the future.

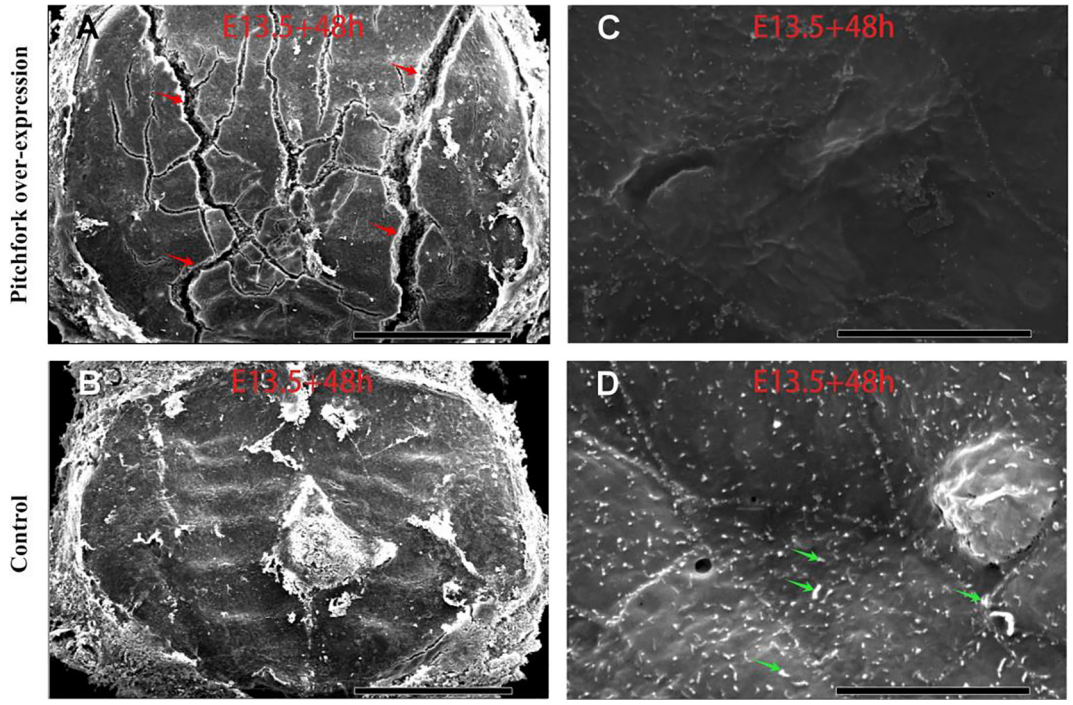

Fig. 1. Scanning electron microscopy (SEM) confirmed the morphological changes in the developing palate after Pitchfork over-expression. A, B lateral cleft palate appears in the Pitchfork over-expression group compare with the control group. C, D Primary cilia was disassembled after Pitchfork over-expression (red arrowheads lateral cleft palate, green arrowheads primary cilia). Bars 500 μm (A, B), 10 μm (C, D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Conflict of interest

The authors do not have any conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.03.005.

References

[1] Novarino G, Akizu N, Gleeson JG. Modeling human disease in humans: the ciliopathies. Cell 2011;147:70–9.
[2] Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. Vertebrate Smoothened functions at the primary cilium. Nature 2005;437:1018–21.
[3] Jin C, Lee JM, Tang Q, et al. Morphological and molecular changes associated with Pitchfork during mouse palate development. Cell Tissue Res 2014;358:385–93.
[4] Manli Z, Yanping L, Yali L. Correlation between primary cilium and Wnt signaling pathway. Hereditas 2015;37:233.
[5] Yi L, Zhi-ming C, Jin-long Z, Peng-fei X, Song W. Research progress of kinesin II family member 3A. Chinese J Tissue Eng Res 2016;20:5617–24.
[6] Kondo S, Sato-Yoshitake R, Noda Y, et al. KIF3A is a new microtubule-based anterograde motor in the nerve axon. J Cell Biol 1994;125:1095–107.
[7] Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV. Hedgehog signalling in the mouse requires intraflagellar transport proteins. Nature 2003;426:83–7.
[8] Kinzel D, Boldt K, Davis EE, et al. Pitchfork regulates primary cilia disassembly and left-right asymmetry. Dev Cell 2010;19:66–77.
[9] Eggenschwiler JT, Anderson KV. Cilia and developmental signaling. Annu. Rev Cell Dev Biol 2007;23:345–73.
[10] Jung B, Padula D, Burtscher I, et al. Pitchfork and Gprasp2 target smoothened to the primary cilium for hedgehog pathway activation. PLoS One 2016;11:e0149477.
[11] Yamazaki H, Nakata T, Okada Y, Hirokawa N. Cloning and characterization of KAP3: a novel kinesin superfamily-associated protein of KIF3A/3B. Proc Natl Acad Sci USA 1996;93:8443–8.
[12] Rosenbaum JL, Witman GB. Intraflagellar transport. Nat Rev Mol Cell Biol 2002;3:813–25.
[13] Marszalek JR, Ruiz-Lozano P, Roberts E, Chien KR, Goldstein LS. Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. Proc Natl Acad Sci USA 1999;96:5043–8.
[14] Brugmann SA, Allen NC, James AW, Mekonnen Z, Madan E, Helms JA. A primary cilia-dependent etiology for midline facial disorders. Hum Mol Genet 2010;19:1577–92.
[15] Christensen ST, Pedersen LB, Schneider L, Satir P. Sensory cilia and integration of signal transduction in human health and disease. Traffic 2007;8:97–109.
[16] Ping Cheng, Chuan-yong Guo, Xuan-fu X. Research progress of the hedgehog signaling pathway target genes and tumor, progress in modern. Biomedicine 2013;13:6393–5.
[17] Tremblay MR, McGovern K, Read MA, Castro AC. New developments in the discovery of small molecule Hedgehog pathway antagonists. Curr Opin Chem Biol 2010;14:428–35.
[18] Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. N Engl J Med 2011;364:1533–43.