Developmental Biology 409 (2016) 55–71

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Primary cilia are critical for Sonic hedgehog-mediated dopaminergic neurogenesis in the embryonic midbrain

Mary Gazea [a,1,2], Evangelia Tasouri [b,c,1], Marianna Tolve [a], Viktoria Bosch [a], Anna Kabanova [a], Christian Gojak [d,e,3], Bahtiyar Kurtulmus [f], Orna Novikov [g], Joachim Spatz [d,e], Gislene Pereira [f], Wolfgang Hübner [h], Claude Brodski [g], Kerry L. Tucker [b,c,i,*], Sandra Blaess [a,**]

[a] Institute of Reconstructive Neurobiology, University of Bonn, 53127 Bonn, Germany
[b] Interdisciplinary Center for Neurosciences, University of Heidelberg, 69120 Heidelberg, Germany
[c] Institute of Anatomy and Cell Biology, University of Heidelberg, 69120 Heidelberg, Germany
[d] Department of Biophysical Chemistry, University of Heidelberg, 69120 Heidelberg, Germany
[e] Department of New Materials and Biosystems, Max Planck Institute for Intelligent Systems, 70569 Stuttgart, Germany
[f] Molecular Biology of Centrosomes and Cilia, German Cancer Research Center, DKFZ-ZMBH Alliance, 69120 Heidelberg, Germany
[g] Department of Physiology and Cell Biology, Zlotowski Center for Neuroscience, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel
[h] Molecular Biophotonics, University of Bielefeld, 33615 Bielefeld, Germany
[i] University of New England, College of Osteopathic Medicine, Department of Biomedical Sciences, Center for Excellence in the Neurosciences, Biddeford, ME 04005, USA

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form
21 October 2015
Accepted 30 October 2015
Available online 2 November 2015

Keywords:
Primary cilia
Ift88
Intraflagellar transport
Midbrain
Dopaminergic neurons
Shh

ABSTRACT

Midbrain dopaminergic (mDA) neurons modulate various motor and cognitive functions, and their dysfunction or degeneration has been implicated in several psychiatric diseases. Both Sonic Hedgehog (Shh) and Wnt signaling pathways have been shown to be essential for normal development of mDA neurons. Primary cilia are critical for the development of a number of structures in the brain by serving as a hub for essential developmental signaling cascades, but their role in the generation of mDA neurons has not been examined. We analyzed mutant mouse lines deficient in the intraflagellar transport protein IFT88, which is critical for primary cilia function. Conditional inactivation of *Ift88* in the midbrain after E9.0 results in progressive loss of primary cilia, a decreased size of the mDA progenitor domain, and a reduction in mDA neurons. We identified Shh signaling as the primary cause of these defects, since conditional inactivation of the Shh signaling pathway after E9.0, through genetic ablation of *Gli2* and *Gli3* in the midbrain, results in a phenotype basically identical to the one seen in *Ift88* conditional mutants. Moreover, the expansion of the mDA progenitor domain observed when Shh signaling is constitutively activated does not occur in absence of *Ift88*. In contrast, clusters of Shh-responding progenitors are maintained in the ventral midbrain of the hypomorphic *Ift88* mouse mutant, *cobblestone*. Despite the residual Shh signaling, the integrity of the mDA progenitor domain is severely disturbed, and consequently very few mDA neurons are generated in *cobblestone* mutants. Our results identify for the first time a crucial role of primary cilia in the induction of mDA progenitors, define a narrow time window in which Shh-mediated signaling is dependent upon normal primary cilia function for this purpose, and suggest that later Wnt signaling-dependent events act independently of primary cilia.

© 2015 Elsevier Inc. All rights reserved.

* Correspondence to: University of New England, College of Osteopathic Medicine, Department of Biomedical Sciences, Center for Excellence in the Neurosciences, 11 Hills Beach Rd., Biddeford, ME 04005, USA. Fax: +1 207 202 5887.
** Correspondence to: University of Bonn, Institute of Reconstructive Neurobiology, Sigmund-Freud-Str. 25, 53127 Bonn, Germany. Fax: +49 6885 501.
E-mail addresses: ktucker2@une.edu (K.L. Tucker), sblaess@uni-bonn.de (S. Blaess).
[1] Equal contribution.
[2] Current adress: Max Planck Institute of Psychiatry, Kraepelinstraße 2-10, 80804 Munich, Germany.
[3] Current address: PromoCell GmbH, Sickingerstr. 63/65, 69126 Heidelberg, Germany.

http://dx.doi.org/10.1016/j.ydbio.2015.10.033
0012-1606/© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Midbrain dopaminergic (mDA) neurons regulate movement, reward behavior, and cognitive function, and their degeneration or dysfunction is implicated in a number of neurological diseases, including Parkinson's disease, schizophrenia, and depression (Albin et al., 1989; Tye et al., 2013; Winterer and Weinberger, 2004). The anatomy and physiology of mDA neurons have been studied extensively, but the mechanisms underlying their development are still not understood. Recent studies conclusively showed that mDA neurons arise from neural progenitors in the ventral midline (floor plate) of the embryonic midbrain (reviewed in (Blaess and Ang, 2015)). These mDA progenitors are defined by the expression of the transcription factors Lmx1a/b (LIM homeobox transcription factor 1 alpha/beta) and Foxa1/2 (forkhead box 1/2), and the secreted morphogen Shh (Sonic Hedgehog) (Andersson et al., 2006; Deng et al., 2011; Lin et al., 2009; Nakatani et al., 2010; Yan et al., 2011). The induction of these factors in the floor plate and subsequent induction of mDA progenitors is dependent on signaling pathways activated by Shh, Wnts, and fibroblast growth factors (Fgf) (Andersson et al., 2006; Andersson et al., 2013; Blaess et al., 2006; Farkas et al., 2003; Fernando et al., 2014; Lahti et al., 2012; Yang et al., 2013; Ye et al., 1998).

Shh is critical for specification of ventral cell fates in many parts of the nervous system. Shh signaling is transduced by the transmembrane receptors patched (Ptch) and Smoothened (Smo) and intracellularly by the Gli zinc-finger transcription factors (Gli1-3). Gli2 is the main activator downstream of Shh signaling; Gli3 acts as a repressor in the pathway (Fuccillo et al., 2006). The formation of both a Gli2 transcriptional activator and a Gli3 transcriptional repressor – and consequently Hedgehog signaling – is critically dependent upon a functional primary cilium in the responding cell (Goetz and Anderson, 2010). Primary cilia are short protrusions from the plasma membrane that are found extending from the surface of most vertebrate cell types (Hoyer-Fender, 2013). The axonemal center of the cilium is composed of ordered microtubule doublets originating in a centrosome-derived basal body. Trafficking of cargo, including signal transduction proteins, into and out of the cilium is a tightly-regulated process dependent upon intraflagellar transport (IFT). IFT utilizes the motor proteins kinesin-II and cytoplasmic dynein-II for anterograde and retrograde transport, respectively, while proteins of the IFT-B and IFT-A classes, respectively, are critical for both transport and signal transduction (Scholey, 2003). Genetic evidence has revealed that multiple mutants in IFT proteins, and in proteins localized to the basal body or the axoneme, demonstrate characteristic defects in Shh signaling in organs throughout the body (Tasouri and Tucker, 2011). Cell biological analysis has shown that crucial components of Hedgehog signaling specifically localize to the cilia in a dynamic fashion, including Ptch1, Smo, and the Gli proteins (Corbit et al., 2005; Haycraft et al., 2005; Rohatgi et al., 2007).

In addition, both canonical and non-canonical Wnt signaling pathways have also been implicated in the functional biology of primary cilia, but the evidence for their absolute dependence upon primary cilia function is controversial. Studies in different organ systems such as the forebrain, skeleton, and abdominal viscera, indicate that the disruption of cilia- or basal body-localized proteins results in dysfunctional Wnt signaling (in terms of beta-catenin or Axin2 levels as a readout for canonical signaling, or defects in convergent extension movements during gastrulation) (Oh and Katsanis, 2012). However, comprehensive studies examining early development in zebrafish and mice lacking primary cilia observed no defects in classic Wnt-dependent developmental processes (Huang and Schier, 2009; Ocbina et al., 2009; Tran et al., 2008). Clearly, abnormalities in Wnt signaling are also observed in progression to cystic kidney disease when proteins localizing to cilia and basal bodies bear mutations (Lienkamp et al., 2012). To reconcile these disparate findings, various possibilities exist, including the idea that Wnt signaling is dependent upon basal body function, but not on IFT or axoneme-localized ciliary processes (Huang and Schier, 2009). Alternatively, subcellular localization of "ciliary" proteins distinct from the primary cilium / basal body may be responsible for modifications in Wnt signaling pathways.

Given that both Shh and Wnt signaling are essential for the specification of mDA progenitors, and that not only Shh, but also Wnt signaling may depend upon primary cilia function, it is of great interest to understand the impact of primary cilia function on mDA specification. Here we address this question using mouse genetic models in which IFT and Shh signaling components are disrupted.

2. Materials and methods

2.1. Mouse lines

All experiments were conducted according to the guidelines of the states of Baden-Württemberg and North Rhine-Westphalia, Germany. *cbbs* (Willaredt et al., 2008), *Ift88*^{flox} (Haycraft et al., 2007), *Gli3*^{Xt} (Hui and Joyner, 1993), *Gli2*^{zfd} (Matise et al., 1998), *Gli2*^{flox} (Corrales et al., 2006), *Gli3*^{flox} (Blaess et al., 2008), *R26*^{SmoM2} (Jeong et al., 2004), and *En1*^{Cre} (Kimmel et al., 2000) alleles were generated as described. 12 noon of the day of the vaginal plug was assigned the date embryonic day 0.5 (E0.5). To generate conditional knockout (cko) mice or mice in which the Shh signaling receptor Smo was conditionally activated (ca), *En1*$^{Cre/+}$ mice were crossed with mice bearing floxed alleles (genotypes in brackets): *Ift88*-cko (*En1*$^{Cre/+}$, *Ift88*$^{flox/flox}$), *Gli2/3*-cko (*En1*$^{Cre/+}$, *Gli2*$^{flox/zfd}$, *Gli3*$^{flox/xt}$), Smo-ca (*En1*$^{Cre/+}$, *SmoM2*$^{flox/+}$) and *Ift88*-cko; Smo-ca (*En1*$^{Cre/+}$, *Ift88*$^{flox/flox}$, *SmoM2*$^{flox/+}$). For all embryonic stages before E12.5, somite-matched embryos were compared for analysis as follows: E9.5: 21-29 somites, E10.5: 35-39 somites; E11.5: >45 somites (Theiler, 1989). Genomic DNA was isolated from embryonic and adult tissue as described (Laird et al., 1991). For PCR genotyping the following primers were utilized Cre-F: 5′-TAAAGATATCTCACGTACTGACGGTG-3′, Cre-R: 5′-TCTCTGACCAGAGTCATCETTAGC-3′, En1WT-F: 5′-CACCGCACCACCAACTTTTTC-3′, En1WT-R: 5′-TCGCATCTGGAGCA-CACAAGAG-3′, Gli3 P1: 5′-GGCCCAAACATCTACCAACACATAG-3′, Gli3 P2: 5′-GTTGGCTGCTGCATGAAGACTGAC-3′, Gli3 P3: 5′-TACCC-CAGCAGGAGACTCAGATTAG-3′, Gli3 P4: 5′-AAACCCGTGGCTCAG-GACAAG-3′, Gli2-S: 5′-AAACAAAGCTCCTGTACACG-3′, Gli2-AS: 5′-CACCCCAAAGCATGTGTTTT-3′, Gli2neo-PA: 5′-ATGCCTGCTCTTTACT-GAAG-3′, Gli2flox-C: 5′-AGGTCCTCTTATTGTCAAG-3′, Gli2flox-D: 5′-GAGACTCCAAGGTACTTAGC-3′, Ift88 common 5′ primer: 5′-GCCTCCTGTTTCTTGACAACA GTG-3′, Ift88 3′ flox and WT allele primer: 5′-GGTCCTAACAAGTAAGCCCAGTGTT-3, R26SMO: R26SMO YFP_F: 5′-CCT CGT GAC CAC CTT CG-3′, R26SMO YFP_R: 5′-TTG ATG CCG TTC TTC TGC-3′.

2.2. Immunohistochemical analysis

Embryos or embryonic brains were dissected, collected in cold 0.1 M phosphate-buffered saline (PBS), and embryonic tail samples were collected separately for DNA extraction and genotyping. The embryos were fixed for periods of 30 min to overnight at 4 °C in 4% paraformaldehyde (PFA) in 0.1 M PBS. After rinsing twice in 0.1 M PBS for 30 min the embryos were either treated in an ascending sucrose series (10, 20, and 30% in 0.1 M PBS) and mounted in Jung tissue freezing medium (Leica Biosystems, Wetzlar, Germany) or dehydrated and processed for paraffin embedding. Stainings of 10–14 μm cryosections or 7 μm paraffin sections were performed as described (Blaess et al., 2011; Brachmann et al., 2007) with the

Download English Version:

https://daneshyari.com/en/article/10931256

Download Persian Version:

https://daneshyari.com/article/10931256

Daneshyari.com