Developmental Biology 395 (2014) 342–354

Contents lists available at ScienceDirect

Developmental Biology

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

Primary cilia function regulates the length of the embryonic trunk axis and urogenital field in mice

Elanor N Wainwright, Terje Svingen, Ee Ting Ng, Carol Wicking, Peter Koopman *

Institute for Molecular Bioscience, The University of Queensland, Brisbane QLD 4072, Australia

ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form
20 August 2014
Accepted 27 August 2014
Available online 16 September 2014

Keywords:
Gonad
Testis
Ovary
Organogenesis
Embryo patterning
Primary cilia

ABSTRACT

The issues of whether and how some organs coordinate their size and shape with the blueprint of the embryo axis, while others appear to regulate their morphogenesis autonomously, remain poorly understood. Mutations in *Ift144*, encoding a component of the trafficking machinery of primary cilia assembly, result in a range of embryo patterning defects, affecting the limbs, skeleton and neural system. Here, we show that embryos of the mouse mutant *Ift144*^{twt} develop gonads that are larger than wild-type. Investigation of the early patterning of the urogenital ridge revealed that the anterior–posterior domain of the gonad/mesonephros was extended at 10.5 dpc, with no change in the length of the metanephros. In XY embryos, this extension resulted in an increase in testis cord number. Moreover, we observed a concomitant extension of the trunk axis in both sexes, with no change in the length of the tail domain or somite number. Our findings support a model in which: (1) primary cilia regulate embryonic trunk elongation; (2) the length of the trunk axis determines the size of the urogenital ridges; and (3) the gonad domain is partitioned into a number of testis cords that depends on the available space, rather than being divided a predetermined number of times to generate a specific number of cords.

Introduction

Embryonic development is orchestrated through an orderly and interconnected progression of events in a precise temporal and spatial sequence. Disruptions in the meshwork of events affecting one tissue can alter the patterning of neighbouring organ systems and have "knock-on" effects in the differentiation and function of their neighbours. In the trunk of the embryo, it is unclear which organs align their development and morphology with the embryo axis rather than having more autonomous regulation of their size and shape.

In mice, the urogenital system is derived from the linear and sequential differentiation of three kidney primordia arising from the intermediate mesoderm. At 8.0 dpc, a portion of the intermediate mesoderm on both sides of the midline undergoes mesenchymal-to-epithelial transition to form the Wolffian ducts, which subsequently extend in a posterior direction until they terminate in the cloaca (Bouchard et al., 2002; Grote et al., 2006). The first primordial kidney—the pronephros—degenerates almost as soon as it forms (Bouchard et al., 2002). Subsequently, the second primordial kidney—the mesonephros—differentiates and will go on to form the reproductive tract. The gonad forms on the ventromedial surface of the mesonephros at 10.5 dpc. At the level of the hindlimb, the remaining intermediate mesoderm condenses and interacts with the Wolffian duct to induce the outgrowth of the final definitive kidney, the metanephros (Saxen and Sariola, 1987).

The gonad develops on the surface of the mesonephros between 9.5 and 10.5 dpc, when coelomic epithelial cells proliferate and undergo epithelial-to-mesenchymal transition to form gonadal somatic cells (Karl and Capel, 1998). Primordial germ cells (PGCs) are specified at the proximal end of the epiblast from where they move to the extra-embryonic mesoderm at the base of the allantois at an earlier stage, around 6.5 dpc (Lawson and Hage, 1994; McLaren and Lawson, 2005; Ohinata et al., 2005). From the extraembryonic mesoderm, PGCs migrate to the hindgut endoderm and subsequently traverse the hindgut mesentery between 9.5 and 10.5 dpc to reach their final destination, the genital ridges (Ginsburg et al., 1990). It has been hypothesised that the long and narrow gonadal structure has evolved to act as a "net" to receive germ cells dispersed in the hindgut (Harikae et al., 2013), but how the size and shape of the gonad domain is regulated remains poorly understood.

The primary cilium is an immotile cellular organelle that protrudes from most non-dividing vertebrate cells (Goetz and Anderson, 2010). In humans and mice, mutations leading to either disruption of primary cilium biogenesis or function can have severe developmental consequences resulting in disorders and diseases collectively referred

* Corresponding author. Fax: +61 7 3346 2101.
E-mail address: p.koopman@imb.uq.edu.au (P. Koopman).

http://dx.doi.org/10.1016/j.ydbio.2014.08.037
0012-1606/

to as ciliopathies. This large, heterogeneous group of disorders includes polycystic kidney disease and retinal pigmentosa, as well as more complex syndromes with variable phenotypes such as Bardet-Biedl, Jeune, short rib polydactyly and Sensenbrenner syndromes. Frequent phenotypes associated with primary cilium defects include skeletal abnormalities, renal cysts, retinal degeneration and neurological defects (Goetz and Anderson, 2010).

In addition to having a mechanosensory role, primary cilia act as a signalling hub and are functionally linked to the Hh, WNT, NOTCH, PDGFRα, FGF and HIPPO pathways (Corbit et al., 2005; Ezratty et al., 2011; Habbig et al., 2012; Neugebauer et al., 2009; Schneider et al., 2005). Proteins are dynamically trafficked up and down the microtubule-based ciliary axoneme using a specialised motor-driven trafficking process known as intraflagellar transport (IFT) (Pazour et al., 2000). Generally, a group of anterograde trafficking proteins (IFT-B) move cargo to the tip of the cilium using a kinesin motor protein complex, whereas retrograde trafficking proteins (IFT-A) move cargo from the tip to the base via a dynein motor protein complex (Ou et al., 2005).

The IFT-A gene *Ift144* (or *Wdr19*) has been linked to both Jeune asphyxiating thoracic dystrophy syndrome and Sensenbrenner or cranio-ectodermal dysplasia syndrome (Bredrup et al., 2011; Fehrenbach et al., 2014). Previous analysis of a mouse model with a hypomorphic missense mutation of *Ift144* ("twinkle-toes", or *Ift144*^{twt}), revealed a number of characteristics of the cognate human diseases, including polydactyly, short rib cage, limb truncation, neural patterning and cleft lip/palate (Ashe et al., 2012; Liem et al., 2012). Rib defects in *Ift144*^{twt} mouse mutants were shown to result from early disrupted somitic patterning in the inter-limb region. In addition, *Ift144*^{twt} embryos have fewer primary cilia, but only very subtle changes to the structure of those primary cilia that do form. Furthermore, embryonic fibroblasts derived from *Ift144*^{twt} embryos display an attenuated response to upstream activation of Hh signalling, whereas analysis *in vivo* revealed a ligand-independent expansion of Hh signalling in some contexts (Ashe et al., 2012; Liem et al., 2012).

In the present study we show that, in addition to the defects previously described, *Ift144*^{twt} mutants display hyperplastic gonads in both XX and XY embryos. Underlying this significant increase in gonad size, *Ift144*^{twt} mice had an early anterior expansion of the gonad domain along the anterior-to-posterior axis, concomitant with an extension in the length of the embryo trunk. These findings help to define the underlying mechanisms of gonad morphogenesis and dependency of organ patterning on the embryonic axis.

Materials and methods

Mice

Ift144^{twt} mice have been described previously and were analysed on a FVB/NJ background (Ashe et al., 2012) and were compared to wild-type littermates. Oct4-GFP strain has also been reported previously (Szabo et al., 2002). For detection of primary cilia in embryonic gonad development, wild-type embryos were collected from timed matings of outbred CD1 strain mice. Mice were staged with noon of the day on which the mating plug was observed designated 0.5 days post-coitum (dpc) and 8–10 tail somite (ts) correlating to 10.5 dpcdpc and 17–18 ts correlating to 11.5 dpc. Sx PCR was used to determine the sex of the embryos (McFarlane et al., 2013). Protocols and use of animals were approved by the Animal Ethics Committee of the University of Queensland, which is registered as an institution that uses animals for scientific purposes under the Queensland Animal Care and Protection Act (2001).

Immunofluorescence

The following primary antibodies against endogenous mouse antigens were used in immunofluorescence; rabbit anti-MVH (code 13840; Abcam) used at 1:400 dilution; mouse anti-E-cadherin (code 610182, Becton Dickinson) used at 1:200 dilution; mouse anti-SOX9 (code H00006662-M01; Abnova) used at 1:200 dilution; rabbit anti-FOXL2 (Polanco JC 2010) used at 1:600 dilution; mouse anti-ARL13B (code 75-287; Antibodies Incorporated) used at 1:200 dilution; mouse anti-OCT4 (code sc-5279; Santa Cruz) used at 1:100 dilution; goat anti-AMH (code SC-5279; Santa Cruz); chicken anti-GFP (code 13970; Abcam) used at 1:400 dilution; goat anti-GATA4 (code sc-1237; Santa Cruz) used at 1:100 dilution, rabbit anti-PAX2 (code 71–6000; Invitrogen) used at 1:200 dilution, Mouse anti-MVH (code ab27591; Abcam) used at 1:600 dilution and rabbit anti-STRA8 (code ab49405; Abcam) used at 1:200 dilution. The secondary antibodies used were donkey anti-goat Alexa 488 (code A11055; Invitrogen) at 1:200 dilution; goat anti-rabbit Alexa 594 (code A11034; Invitrogen) at 1:200 dilution; donkey anti-rabbit Alexa 568 (code A10042; Invitrogen) at 1:200 dilution; and 40,6-diamidino-2-phenylindole (DAPI; 2 ng/μl in PBS; Molecular Probes) at 1:1000 dilution to visualise nuclear DNA in immunofluorescence.

For section immunofluorescence, 7 μm paraffin sections were processed as described previously (Polanco JC 2010). Slides were imaged using a confocal microscope (LSM 510 Meta; Zeiss). For whole-mount immunofluorescence, dissected gonads/mesonephroi were fixed in 4% paraformaldehyde (PFA) in PBTX (PBS containing 0.1% Triton X-100) overnight at 4 °C. Samples were washed in phosphate buffered Saline (PBS) and stored in 100% methanol (MeOH) at −20 °C until required. Samples were rehydrated through at MeOH series and then blocked for 4 h at room temperature in 10% heat inactivate horse serum (HS)/PBTX. Primary antibody was incubated overnight at 4 °C and then washed at least three times in PBTX for a minimum of 24 h. Subsequently, the secondary antibody was incubated overnight at 4 °C diluted in 10% HS/PBTX and then washed three times in PBTX for a minimum of 24 h. Samples were dehydrated into 100% methanol before being cleared by 1:2 benzyl alcohol/benzyl benzoate (BABB). Samples were mounted in a glass bottom dish (code P35G-1.5-14-C, MatTek corporation) and imaged using an inverted LSM 510 Meta (Zeiss) confocal microscope.

Image capture, analysis and processing

For wholemount immunofluorescence, serial non-overlapping Z-slice optical sections were captured for the depth of the sample. For 13.5 dpc XY gonads, samples were imaged on 10× objective at 7.1 μm intervals. For 10.5 dpc urogenital ridges, samples were imaged on a 10x objective with a 0.8× optical zoom, tiled over two different fields of view at 7.1 μm intervals. Optical sections were processed in Imaris software (Bitplane) to produce maximum intensity projections and testis cord 3D models with the rendering tool: surfaces. Whole-mount pictures of embryos and dissected tissue were captured on Olympus SZX-12 stereomicroscope.

Image quantification

Number of somites and embryo lengths were quantified on *Ift144*^{twt} and wild-type littermate control embryos previously stained by *in situ* hybridisation to reveal somite markers (Ashe et al., 2012). For measuring embryo dimensions and urogenital ridge lengths, samples were imaged in the same orientation at the same magnification and then the length of each sample was measured using drawing tools in ImageJ software. Testis cords were counted from the coelomic view of rendered testis cord models. For quantification of number of cells in the testis, the one sagittal section through the centre of the testis (the largest plane)