Biochemical and Biophysical Research Communications 410 (2011) 164–169

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Geminin is required for left–right patterning through regulating Kupffer's vesicle formation and ciliogenesis in zebrafish

Sizhou Huang [a,b,1], Jun Ma [a,b,1], Xiaolin Liu [a,b], Yaoguang Zhang [a], Lingfei Luo [a,b,*]

[a] Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, Chongqing 400715, China
[b] Laboratory of Molecular Developmental Biology, School of Life Sciences, Southwest University, Tiansheng Road 2, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 23 March 2011
Available online 23 April 2011

Keywords:
Geminin
Left–right asymmetry
Kupffer's vesicle
Ciliogenesis
nodal/spaw

ABSTRACT

Geminin plays an important role in coordinating the cell cycle with anterior–posterior patterning during embryonic development. However, whether it is involved in the regulation of left–right (LR) patterning remains unknown. Here, we reported that geminin is required for setting up heart and visceral laterality during zebrafish development. Defective heart and visceral laterality was observed in geminin morphants. Further study demonstrated that the left-sided *nodal/spaw* in the lateral plate mesoderm (LPM) as well as the sideness of its downstream targets *lefty2* and *lefty1* was perturbed in geminin morphants. Upstream of the left-sided Nodal signal along the regulatory cascade of LR asymmetry, knock down of geminin resulted in defective Kupffer's vesicle (KV) formation and ciliogenesis rather than middle line defects. Predominant distribution of an antisense morpholino against geminin in dorsal forerunner cells (DFCs) led to defective KV morphogenesis and perturbed LR asymmetry, similar to those of geminin morphants, indicating a cell-autonomous role of geminin in regulating KV formation and ciliogenesis. Our results demonstrate that geminin is required for proper KV formation and ciliogenesis, thus playing an important part in setting up LR asymmetry.

1. Introduction

Heart and visceral organs such as liver, pancreas, and gut exhibit obvious LR asymmetry in vertebrates [1–4]. In zebrafish, the function of KV/cilia in the determination of LR patterning is similar to that of the node in mouse and chick, which generates directional fluid flow to initiate LR asymmetric signals [5–8]. KV arises from dorsal forerunner cells (DFCs), which are positioned close to the embryonic shield during gastrulation, remained at the leading edge of the epiblast during epiboly, and later move deep into the embryo to form KV [6]. A number of factors and signaling pathways required for LR asymmetry have been reported to regulate the fate of DFCs, KV formation, and ciliogeneis [9–12]. Other factors, such as Fgf8, are not involved in KV formation, but required for proper ciliogenesis [13–16], thus contributing to the determination of LR asymmetry. Midline barriers are functional either within the organizer itself or within differentiated derivatives of the midline organizer such as notochord and floor plate to block the transfer of the left side determinants, ensuring proper LR patterning [17,18]. In zebrafish mutants *no tail*, *floating head*, and *bozozok* that perturb midline formation, there are increases in the incidence of defective LR asymmetry and bilateral expressions of the left-sided genes in the LPM [17,19]. Downstream of KV formation and ciliogenesis, a critical step of LR patterning is the left-sided expression of *nodal/southpaw* (*spaw*) in the LPM, which is conserved in vertebrates [20,21].

Geminin plays a number of roles in the cell cycle regulation and embryonic development. It binds to and inhibits the licensing factor Cdt1 to ensure the fidelity of DNA replication [22,23]. Geminin is enriched in several types of progenitors and undifferentiated cells in early embryo, and also functional as a coordinator between the cell cycle and embryonic patterning [24–28]. Although geminin has been reported to be involved in a number of developmental processes, its role in the determination of LR asymmetry remains to be elucidated.

Here, we used zebrafish as the model system to study the role of geminin in LR patterning. We found that *geminin* expressed in and around KV. Its knock down led to defective heart and visceral laterality as well as perturbation of the left-sided Nodal signal in the LPM. These defects were caused by the requirement of geminin for proper KV formation and ciliogenesis. In addition, the role of

Abbreviations: cha, charon; DFCs, dorsal forerunner cells; hpf, hours post fertilization; KV, Kupffer's vesicle; LPM, lateral plate mesoderm; LR, Left–right; MO, antisense morpholino oligo; ntl, no tail; spaw, southpaw; TUNEL, terminal transferase UTP nick end labeling.

* Corresponding author at: Laboratory of Molecular Developmental Biology, School of Life Sciences, Southwest University, Tiansheng Road 2, Chongqing 400715, China. Fax: +86 23 68367958.
E-mail address: lluo@swu.edu.cn (L. Luo).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2011 Published by Elsevier Inc.
doi:10.1016/j.bbrc.2011.04.085

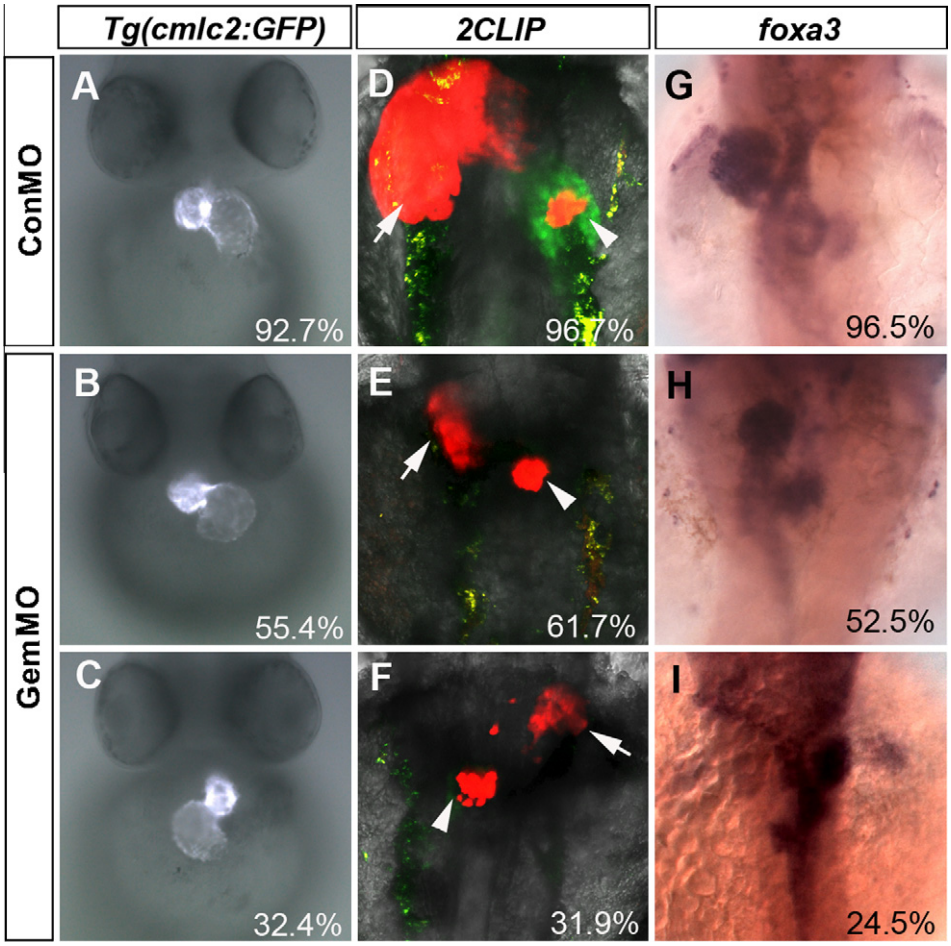

Fig. 1. Defective heart and visceral laterality in geminin morphants. (A–C) 92.7% of control embryos injected with ConMO displayed normal D-loop heart (A). 55.4% and 32.4% of geminin morphants displayed D-loop (B) and reversed L-loop hearts (C), respectively. *Tg(cmlc2:GFP)* transgenic embryos were observed at 53 hpf. (D–F) 96.7% of control embryos displayed visceral *situs solitus* (D). 61.7% and 31.9% of geminin morphants displayed visceral *situs solitus* (E) and *situs inversus* (F), respectively. *2CLIP* transgenic embryos were observed at 76 hpf. (G–I) 96.5% of control embryos displayed visceral *situs solitus* using *foxa3* probe (G). In contrast, 52.5% and 24.5% of geminin morphants displayed visceral *situs solitus* (H) and *situs inversus* (I), respectively. *In situ* hybridized embryos were imaged at 52 hpf. Arrowheads mark the pancreas and arrows mark the liver.

geminin in regulating KV formation and ciliogenesis is cell-autonomous in DFCs.

2. Material and methods

2.1. Zebrafish strains

Zebrafish (*Danio rerio*) of the AB genetic background, *2CLIP* [29], *Tg(cmlc2:GFP)* [30] transgenic lines were raised and maintained under standard laboratory conditions. The developmental stages were characterized as previously described [31].

2.2. Morpholinos and mRNA

Geminin mRNA was synthesized from a linearized plasmid template using the Message Machine Kit (Ambion). Antisense morpholino oligos against *geminin* (GemMO: 5′-CTTTGGTCTTCTGATGG AACTCATA-3′; 500 μM) [27]; *p53* (p53MO: 5′-GCGCCATTGCTTTGC AAGAATTG-3′; 250 μM) [32]; and a control morpholino (ConMO: 5′-CCTCTTACCTCAGTTACAATTTATA-3′; 500 μM) were applied.

2.3. Injections

In order to achieve predominant distribution of GemMO in DFCs, a fluorescein labeled GemMO was injected at the 256–1000 cell stage as previously described [11]. Then, positive embryos were sorted out at the 8–10 h post fertilization (hpf).

2.4. Whole mount in situ hybridization, immunostaining, and TUNEL assay

Whole mount *in situ* hybridization was performed as previously described [27], using established antisense probes [9,10,13]. Whole mount immunostaining was performed to detect cilia in KV using anti-acetylated Tubulin antibodies (Sigma–Aldrich) as previously described [16].

Cell death was detected by terminal transferase UTP nick end labeling (TUNEL) assay as previously described using the Dead-End™ Fluorometric TUNEL System (Promega) [33].

3. Results and discussion

3.1. Geminin is involved in the determination of LR asymmetry

Whole mount *in situ* hybridization indicated an expression of *geminin* in and around KV at the 3–4 somite stage (Supplementary Fig. 1B and C), implying an involvement of geminin in the regulation of LR patterning during embryonic development. In order to analyze this possibility, geminin was knocked down by a specific antisense morpholino oligo (GemMO) [27], and the consequent