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A potential molecular pathogenesis of cardiac/laterality defects in Oculo-Facio-Cardio-Dental syndrome

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ABSTRACT

Pitx2 is the last effector of the left–right (LR) cascade known to date and plays a crucial role in the patterning of LR asymmetry. In *Xenopus* embryos, the expression of *Pitx2* gene in the left lateral plate mesoderm (LPM) is directly regulated by Xnr1 signaling, which is mediated by Smads and FoxH1. Previous studies suggest that the suppression of *Pitx2* gene in the left LPM is a potential cause of cardiac/laterality defects in Oculo-Facio-Cardio-Dental (OFCD) syndrome, which is known to be caused by mutations in *BCL6 co-repressor* (*BCOR*) gene. Recently, our work has revealed that the BCL6/BCOR complex blocks Notch-dependent transcriptional activity to protect the expression of *Pitx2* in the left LPM from the inhibitory activity of Notch signaling. These studies indicated that uncontrolled Notch activity in the left LPM caused by dysfunction of BCOR may result in cardiac/laterality defects of OFCD syndrome. However, this Notch-dependent inhibitory mechanism of *Pitx2* gene transcription still remains unknown. Here we report that transcriptional repressor ESR1, which acts downstream of Notch signaling, inhibits the expression of *Pitx2* gene by binding to a left side-specific enhancer (ASE) region in *Pitx2* gene and recruiting histone deacetylase 1 (HDAC1) to this region. Once HDAC1 is tethered, histone acetyltransferase p300 is no longer recruited to the Xnr1-dependent transcriptional complex on the ASE region, leading to the suppression of *Pitx2* gene in the left LPM. The study presented here uncovers the regulatory mechanism of *Pitx2* gene transcription which may contribute to an understanding of pathogenesis of OFCD syndrome.

Introduction

Anatomical LR asymmetry of the internal organs, such as the orientation of the cardiovascular system, visceral organs and the number of lung lobes, is conserved in vertebrates (Blum et al., 2009; Levin, 2005; Palmer, 2004). Although the mechanisms involved in breaking LR symmetry during very early vertebrate development may not be conserved, the left-side specific expression of genes in the LPM such as *Xnr*1(a *Xenopus* ortholog of mouse *Nodal*), *Lefty* as well as *Pitx*2 have been observed in all vertebrates studied to date and these factors are essential for the patterning of LR asymmetry (Boorman and Shimeld, 2002; Hamada et al., 2002; Kato, 2011; Raya and Belmonte, 2006; Speder et al., 2007).

Pitx2 is a homeobox transcription factor that plays an important role for establishing LR asymmetry during development. In fact, knockout of Pitx2 in mice results in severe cardiac/laterality defects including transposition of the great arteries, double-outlet right ventricle, septal defect, right cardiac isomerism and right lung isomerism (Gage et al., 1999; Lin et al., 1999; Lu et al., 1999). Furthermore, ectopic expression of Pitx2 in the right side of chick, zebrafish and frog embryos affected the direction of heart looping and gut coiling (Essner et al., 2000; Logan et al., 1998; Ryan et al., 1998). During LR patterning, Pitx2 acts downstream of TGFβ superfamily Xnr1 in the left LPM of *Xenopus* embryos (Campione et al., 1999; Schweickert et al., 2000) and this molecular cascade in the left LPM is conserved in all vertebrates examined to date (Long et al., 2003; Meno et al., 1998; Piedra et al., 1998; Yoshioka et al., 1998). The Xnr1 signaling pathway, which induces the expression of *Pitx*2 in the left LPM, is mediated by the transcriptional complex including Smad2/3, Smad4 and FoxH1. Transcription factor FoxH1 directly binds to the ASE region in *Pitx*2 gene and initiates transcription of *Pitx*2 gene (Shiratori et al., 2001). Interestingly, previous studies indicated that the suppression of *Pitx*2 in the left LPM induced by dysfunction of BCOR may be a cause of laterality defects in the heart and other viscera of patients with OFCD syndrome (Hilton et al., 2007; Lin et al., 2000).

OFCD syndrome is an X-linked disorder characterized by ocular, dental, cardiac/laterality and skeletal anomalies as well as mental retardation (Aalfs et al., 1996; Gorlin et al., 1996; Hayward, 1980; Hilton et al., 2007; Lin et al., 2000; Marashi and Gorlin, 1990, 1992;

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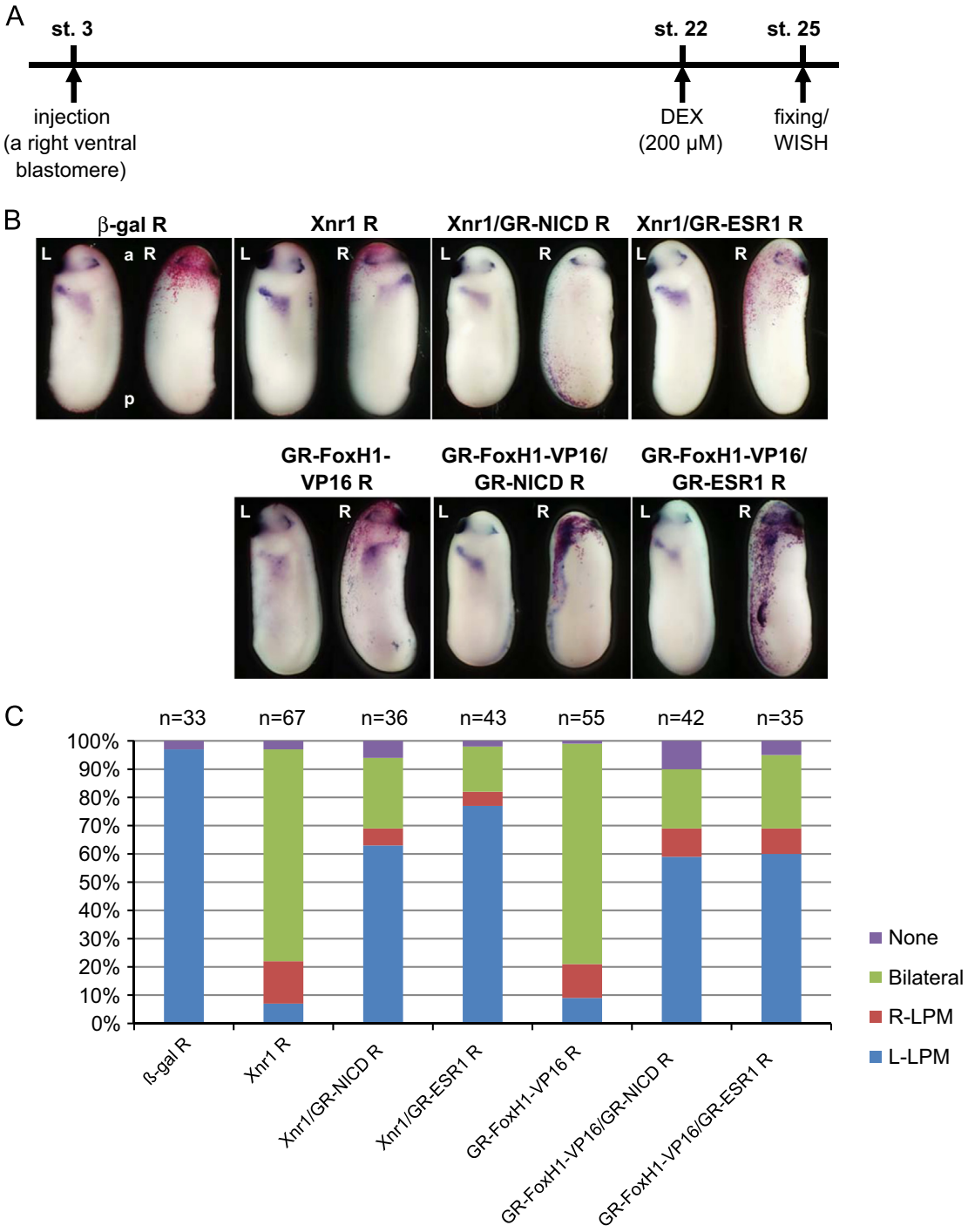

Fig. 1. The Xnr1-dependent transcriptional complex is a potential target of Notch signaling. (A) The experimental strategy. (B) One pg Xnr1 or 50 pg GR-FoxH1-VP16 RNA were injected into a right ventral blastomere of 4-cell-stage embryos with or without 2 ng GR-NICD or 1 ng GR-ESR1 RNA. DEX was added into the culture medium at stage 22. The expression of *Pitx2* at stage 25 was tested by whole mount in situ hybridization. (C) The quantitative assessment of the injections in (B). At least three independent experiments were performed. "*n*" indicates the number of injected embryos. R: Injection into the right side of embryo.

Wilkie et al., 1993). Frequent ocular defects include congenital cataracts and microphthalmia. Facial anomalies include septate nasal tip, high nasal bridge, midface hypoplasia as well as palatal anomalies. Congenital cardiac abnormalities comprise septal defects and mitral valve defects. Dental irregularities include canine radiculomegaly, delayed and persistent dentition as well as hypodontia. Skeletal anomalies contain syndactyly and hammer-type flexion deformities. Defective lateralization includes dextrocardia, asplenia and intestinal malrotation. Genetic studies have shown that mutations in *BCOR* gene at chromosomal location Xp11.4 cause OFCD syndrome (Ng et al., 2004). These mutations in *BCOR* gene result in premature termination of the protein with deletion of the C-terminal domain (Horn et al., 2005; Ng et al., 2004; Oberoi et al., 2005). BCOR was originally identified as a co-repressor of transcriptional repressor BCL6 (Huynh et al., 2000). BCOR has been reported to interact with histone deacetylase (HDAC), demethylase and H2A ubiquitin ligase (Gearhart et al., 2006; Huynh et al., 2000; Sanchez et al., 2007; Tsukada et al., 2006), suggesting that BCOR may mediate epigenetic silencing to repress transcription of target genes. Therefore, uncontrolled