The Loss of Foxc2 Expression in the Outflow Tract Links the Interrupted Arch in the Conditional Foxc2 Knockout Mouse

27

Mohammad Khaja Mafij Uddin, Wataru Kimura, Mohammed Badrul Amin, Kasumi Nakamura, Mohammod Johirul Islam, Hiroyuki Yamagishi, and Naoyuki Miura

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Congenital heart disease is the most common birth defects, affecting 1 % live births [1]. The cardiovascular system undergoes a series of morphogenetic events to form a heart and an aorta in fetuses. Formation of the heart and aorta requires migration, differentiation, and precise interactions among multiple cells from several embryonic origins [2]. Forkhead box2 (Foxc2) encodes a transcription factor and is expressed in mesodermal tissues, such as the pharyngeal artery, outflow tract endothelial/surrounding mesenchyme, bone, and kidney [3]. Simple knockout of Foxc2 in mouse causes an interrupted aortic arch, ventricular septal defect, cleft palate, and skeletal malformation [4]. The heart is made from primary and secondary heart field progenitors. The primary heart field gives rise to the left ventricle and atria, while the secondary heart field contributes mainly to the right ventricle and outflow tract [5] (Fig. 27.1).

To explore the tissue-specific roles of Foxc2 in aortic arch remodeling, we generated mice carrying a floxed allele of *Foxc2* ($Foxc2^{flox}$) and crossed them with several Cre mice, including the primary heart field (Nkx2.5-Cre knock-in)-specific and secondary heart field (Islet1-Cre knock-in and Tbx1-Cre transgenic)-

M.K.M. Uddin • M.B. Amin • K. Nakamura • M.J. Islam • N. Miura (✉)
Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu, Japan
e-mail: nmiura@hama-med.ac.jp

W. Kimura
Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu, Japan

Division of Cardiology, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA

H. Yamagishi
Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

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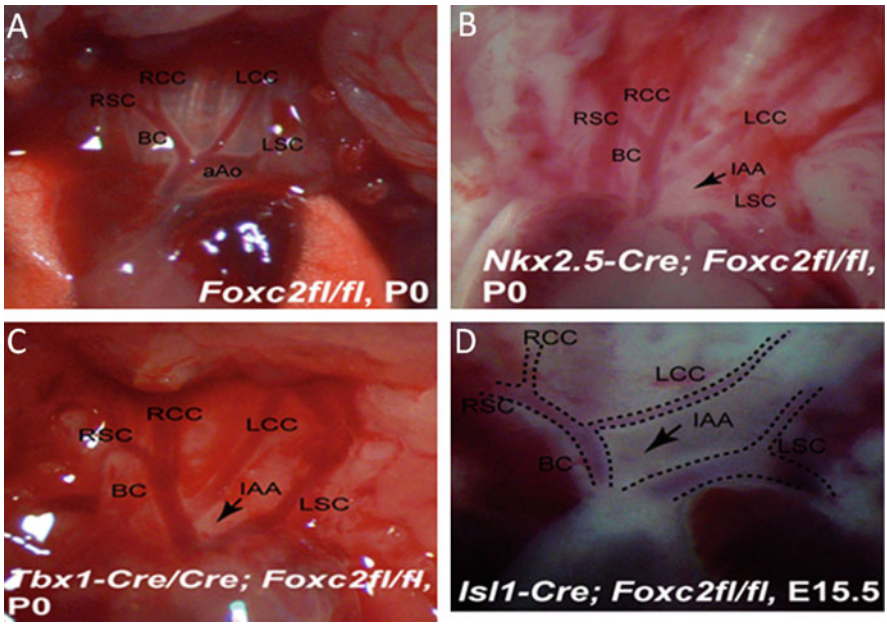

Fig. 27.1 Aortic arch abnormalities in Foxc2 conditional knockout mice. (**a**) Normal aortic arch formation in control mice ($Foxc2^{flox/flox}$). (**b–d**) Conditional knockout mice showed interrupted aortic arch (IAA) type B where part of the aorta between LCC and LSC is missing (*arrow*). *LCC* left common carotid artery, *LSC* left subclavian artery, *aAo* arch of the aorta, *Pt* pulmonary trunk, *RCC* right common carotid artery, *RSC* right subclavian artery, *BC* brachiocephalic artery

specific Cre lines. Surprisingly, conditional knockout (cKO) of *Foxc2* in the primary heart field (Nkx2.5-Cre;$Foxc2^{flox/flox}$) and secondary heart field (Islet1-Cre;$Foxc2^{flox/flox}$ and Tbx1-Cre;$Foxc2^{flox/flox}$) resulted in an interrupted aortic arch and perinatal lethality in mice. X-gal staining and immunostaining with anti-Foxc2 antibody confirmed that Foxc2 expression in the aortic arch was intact but deleted in the outflow tract in these cKO embryos. These results indicate that the Foxc2 expression in the outflow tract, rather than direct role in the aortic arch, is crucial for the aortic arch remodeling. It assumed that Foxc2 in the outflow tract regulates aortic arch remodeling via secreted factors such as Fgf8, Fgf10, and other genes.

References

1. Bruneau BG. The developmental genetics of congenital heart disease. Nature. 2008;451:943–8.
2. Olson EN, Srivastava D. Molecular pathways controlling heart development. Science. 1996;272:671–6.
3. Miura N, Wanaka A, et al. MFH-1, a new member of the fork head domain family, is expressed in developing mesenchyme. FEBS Lett. 1993;326:171–6.
4. Iida K, Miura N, et al. Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. Development. 1997;124:4627–38.
5. Kelly RG, Buckingham ME. The anterior heart-forming field: voyage to the arterial pole of the heart. Trends Genet. 2002;18:210–6.