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Exploring a novel molecular mechanism underlying the cardiac development implicated in the outflow tract defects

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The genetic basis of most congenital heart defects (CHD) is still largely unknown. A genetic interaction of two progenitor cell lineages, the cardiac neural crest (CNC) and the second heart field (SHF), play key roles in development of the cardiac outflow tract (OFT). In order to explore molecular mechanisms underlying OFT defects, we analyzed genes essential for CNC and SHF, using DNA from patients with OFT defects, and identified a transcription factor GATA6 as a novel genetic cause of OFT defects. We also demonstrated that Gata6 directly regulated the expression of Semaphorin 3C (Sema3c) that mediates a neurovascular guiding signaling essential for the interaction between CNC and SHF, and that mutations in *GATA6* disrupted its direct regulation of *SEMA3C*, resulting in OFT defects. To further clarify the regulatory mechanism of *Sema3c*, we delineated putative promoter/enhancer sequences of *Sema3c* and identified conserved regulatory elements for Fox and Sox factors in the 5'UTR region, and for T-box factors in the 3'UTR region, respectively. Among Fox, Sox and T-box transcription factors, Foxc1, Foxc2, Sox4 and Tbx1 have been shown to play essential roles in the OFT development. Interestingly, Foxc1, Foxc2 and Sox4 showed positive transactivation of *Sema3c* through direct bindings to their regulatory elements. Moreover, Tbx1 negatively regulated the transactivation of *Sema3c* by Gata6, probably via its direct binding to the regulatory elements on the 3'UTR sequence of *Sema3c* along with its direct interaction with Gata6. Consistent with these results, in transgenic mice where the *lacZ* reporter is expressed under control of the 3'UTR enhancer of *Sema3c*, the *lacZ* expression was upregulated and ectopically expanded to whole pharyngeal mesenchyme in mice with *Tbx1* hypomorphic alleles, while it was restricted to the SHF in the pharyngeal mesoderm of wild-type mice. These results suggest a novel molecular mechanism underlying the differentiation of OFT progenitor cells where Tbx1 may restrict the *Sema3c* expression to progenitor cells in the SHF and Gata6, Foxc1/c2 and Sox4 may activate the *Sema3c* expression in these cells in a process of their migration and differentiation into OFT myocardium, eventually leading to a proper signal for the CNC migration into the OFT.