Combinatorial Functions of Transcription Factors and Epigenetic Factors in Heart Development and Disease

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Abstract

Heart malformations are the most common type of birth defect, affecting more than 2 % of newborns and causing significant morbidity and mortality. In the past two decades, studies have revealed the function and importance of cardiac transcription factors during heart development and in congenital heart disease. Transcription factors generally form complexes with other transcription factors and/or with chromatin factors to perform specific functions. This review focuses on how chromatin factors modify cardiac transcription factors during cardiovascular development and disease.

Keywords

Cardiac development • Cardiac disease • T-box genes • Epigenetic factors • SWI/SNF-type chromatin remodeling factors

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42.1 Transcription Factors in Heart Development

The heart is an organ that pumps blood to and from the body's tissues through the blood vessels. Cardiac muscle contains cardiomyocytes, which ensure the heart's contractile ability; however, cardiomyocytes alone are not sufficient for the heart to function. Other components, such as the conduction cells, fibroblasts, blood vessels, and endocardial cells, are important for maintaining the heart's systemic pumping ability. Each cell type can be identified by its expression of specific transcription factors, signaling molecules, and/or function-specific proteins (Fig. 42.1). The heart is the first organ to form in vertebrates, and it performs a vital role in distributing oxygen and nutrients throughout the embryo. The primordial heart is derived from cardiovascular mesodermal cells that transiently express *T* (*Brachyury*), *Mesp1*, and *Flk1* during gastrulation. A subset of these cardiac mesodermal cells gives rise to cardiac progenitor cells, which can differentiate into any type of cardiac cell.

T-box transcription factors compose a conserved family of genes that are important for heart development and patterning. In humans, disruption of the cardiac *T-box* genes leads to various congenital heart defects [1]. Mutations in *Tbx5* are associated with Holt-Oram syndrome [2, 3], whereas mutations in the *Tbx20* gene result in atrial septal defect [4, 5]. Interestingly, in the developing heart, Tbx5 and Tbx20 are complementarily expressed in the left and right ventricle, respectively (Fig. 42.2) [6]. The regions in which the *T-box* genes are expressed and the regions that are defective in a given disease are very similar. *Tbx5* is expressed in the inflow tract, atria, AV cushion, and left ventricle but not in the outflow tract or

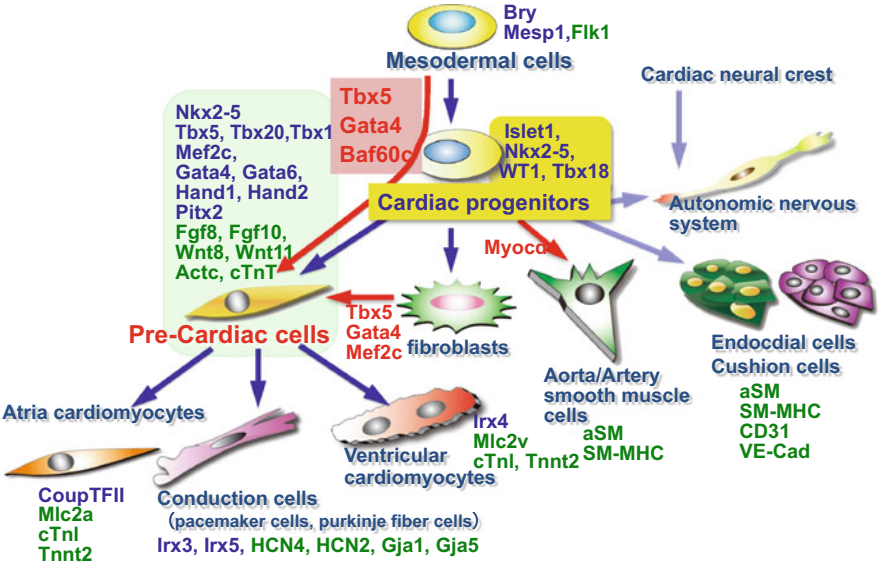

Fig. 42.1 Multiple cardiac cell types. Each cell type differentiates from mesoderm-derived cardiac progenitor cells. The major molecules associated with each cell type are indicated

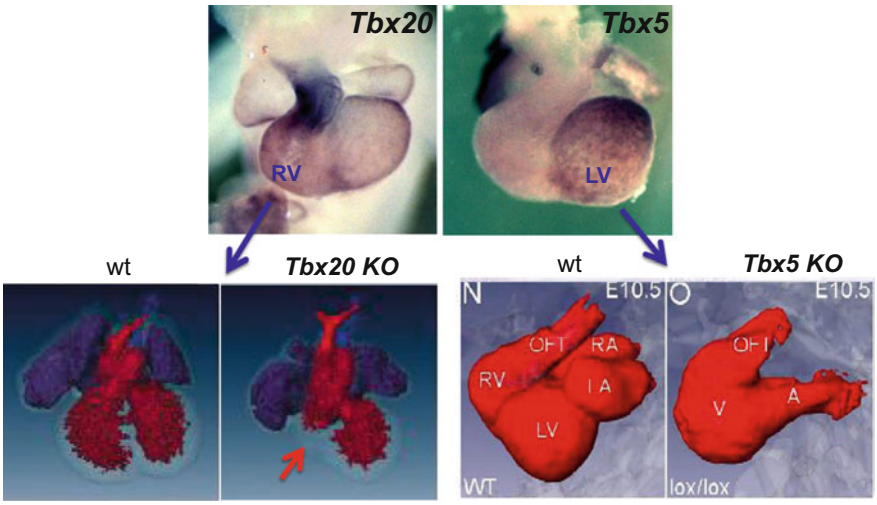

Fig. 42.2 The expression patterns of *Tbx5* and *Tbx20* in the mouse heart. These genes show complementary expression patterns in the ventricles. The knockout mice for each gene show hypoplasia in the same region in which the gene is normally expressed (Adapted from Bruneau et al. [8] and Takeuchi et al. [9])

the right ventricle; *Tbx5* expression appears to be restricted to the first heart field (FHF)-derived region [7]. *Tbx5* knockout mice experience severe left ventricle hypoplasia and die at approximately E9 without their hearts ever beating (Fig. 42.2) [8]. By contrast, *Tbx20* is primarily expressed in the outflow tract and the right ventricle, which are derived from the second heart field (SHF). *Tbx20* knockdown mice develop a single ventricle and show severe hypoplasia of the right ventricle (Fig. 42.2) [9]. These facts indicate that Tbx5 and Tbx20 may specify the identity of each ventricle. Tbx5 also acts in association with Sall4 in ventricular septum formation. Sall4 is a zinc-finger transcription factor that, when mutated, causes Okihiro syndrome (Duane-radial ray syndrome, DRRS) in humans [10, 11]. The heart and limb phenotypes of Okihiro syndrome are very similar to those of Holt-Oram syndrome. In fact, some Holt-Oram patients lack mutations in *TBX5* and instead have mutations in *SALL4* [12]. Tbx5 and Sall4 participate in protein-protein interactions and synergistically regulate downstream gene expression [13]. Furthermore, Tbx5 is a key gene involved in the acquisition of the ventricular septum during vertebrate evolution [14]. During vertebrate evolution from aquatic to terrestrial life, the morphology of the heart has changed. As a result, avian and mammalian hearts contain four chambers—two atria and two ventricles—and their circulatory systems contain two loops, the pulmonary and systemic loops, that separate the oxygen-rich and oxygen-poor blood. In vertebrates with four-chambered hearts, *Tbx5* expression is restricted to the left ventricle, whereas in animals with a single ventricle, *Tbx5* expression is observed throughout the ventricle. Reptiles show a unique *Tbx5* expression pattern that is associated with

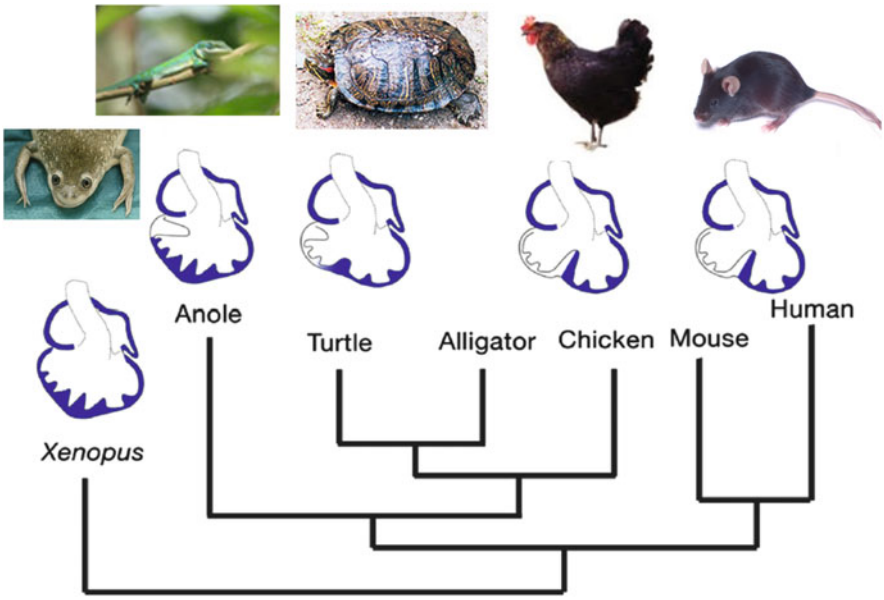

Fig. 42.3 *Tbx5* expression and heart morphology in vertebrates. Note that animals with two ventricles express *Tbx5* on the left side (Adapted from Koshiba-Takeuchi et al. [14])

their ventricular morphology. *Anolis*, which are a type of squamate, have a single ventricle that expresses *Tbx5* throughout this chamber and throughout development. Interestingly, turtles show a left-high to right-low gradient of *Tbx5* expression during late developmental stages, and a septum-like structure forms in the middle of the ventricle (Fig. 42.3). To confirm the precise interaction between *Tbx5* expression patterns and ventricular septum formation, we performed Tbx5 mis-expression experiments using transgenic mice. Transgenic mice that express Tbx5 throughout the ventricle fail to form a ventricular septum. These results strongly indicate that *Tbx5* expression in the left ventricle is important for the development of two-chambered ventricles. We hypothesized that the regulatory region of *Tbx5* might have been modified during vertebrate evolution, thereby changing the *Tbx5* expression pattern and the ventricular morphology, as shown in Fig. 42.3.

42.2 Chromatin Factors and Cardiac Differentiation

Recent studies have shown that chromatin factors are essential for determining cell fate in several organs. In heart development, SWI/SNF-type chromatin remodeling factors play key roles in the differentiation of cardiomyocytes by interacting with heart-specific transcription factors [15, 16]. Cardiac transcription factors alone are not sufficient to induce cardiomyocyte differentiation in vivo or in vitro (Figs. 42.4 and 42.5). This result suggests that chromatin accessibility is important for

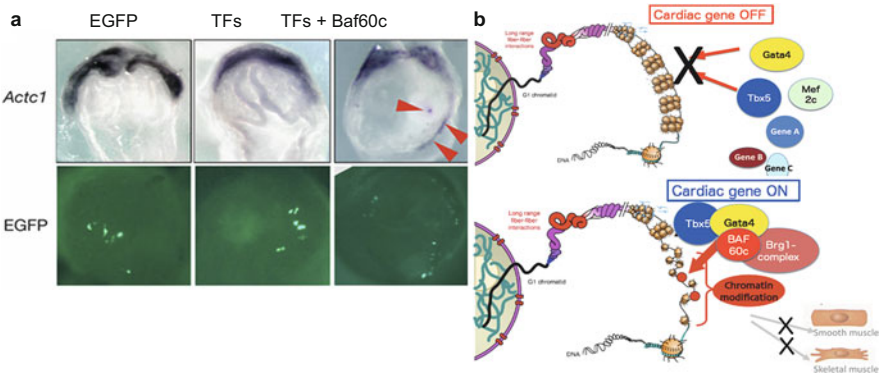

Fig. 42.4 (**a**) A mixture of transcription factors (*TFs*), Baf60c and EGFP, but not the control (EGFP) or TFs + EGFP, ectopically induce *Actc1* in the lateral plate mesodermal region. (**b**) A schematic of cardiac gene regulation. The SWI/SNF complex-mediated change in chromatin conformation is important for the activation of cardiac gene transcription (Adapted from Takeuchi and Bruneau [18] and Van Weerd et al. [15])

Tbx5	Gata4	Gata1	Nkx2-5	Baf60c	Baf60b	*Actc1,Myl7* expression	Beating
+	−	−	−	−	−	×	×
−	+	−	−	−	−	×	×
−	−	+	−	−	−	×	×
+	+	−	−	−	−	×	×
+	−	+	−	−	−	×	×
−	−	−	+	−	−	×	×
+	−	−	+	−	−	×	×
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−	+	−	−	+	−	○	×
−	+	−	−	−	+	○	×
−	−	+	−	+	−	○	×
−	−	+	−	−	+	×	×
+	+	−	+	+	−	○	○
+	+	−	−	+	−	○	○

Fig. 42.5 Schematic diagram shows that ectopic expression of major cardiac contracted genes (Actc1 and Myl7) are observed by combinatorial transfection of cTF and Baf60c into ex vivo mouse

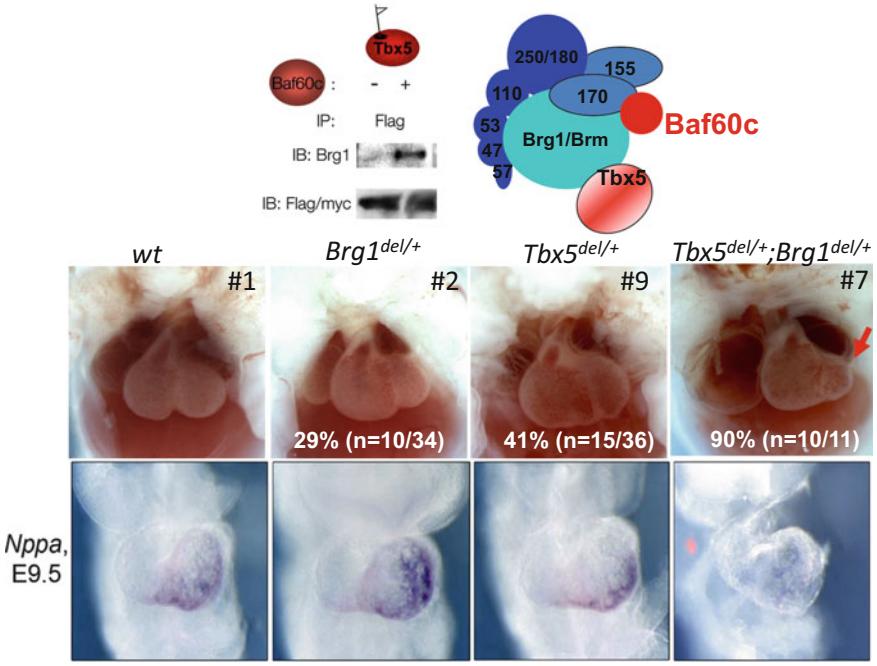

Fig. 42.6 The combinatorial functions of cardiac transcription factors and Baf chromatin remodeling factors. Only the transcription factors can induce cardiac markers, but the differentiation of beating cardiomyocytes requires both transcription factors and chromatin remodelers

transcription factors to bind to their target sites. Therefore, we searched for chromatin remodeling factors that are expressed in the cardiac region at early stages of heart development. A previous study showed that a component of the SWI/SNF-type chromatin remodeling complex, Baf60c (also known as *Smarcd3*), has specific roles in heart development [17]. When a mixture of cardiac transcription factors and Baf60c was injected into the lateral plate mesoderm of mouse embryos, alpha cardiac actin-positive cells were ectopically induced (Fig. 42.4) [18]. These ectopically induced cardiac cells could beat, which showed that they were functional cardiomyocytes. Baf60c can directly associate with the Tbx5, Nkx2-5, Gata4, and RBPjk proteins and regulate the transcription of downstream genes [17, 19]. Mutations in chromatin factors cause abnormal cardiac function in both mice and humans. Baf60c determines a cell's fate by not only loosening the chromatin structure but also synergistically interacting with specific factors. When associated with Tbx5, Brg1, a core protein of the SWI/SNF-type chromatin remodeling complex, synergistically regulates cardiac differentiation in the presence of Baf60c [20]. Mice heterozygous for both *Brg1* and *Tbx5* had more severe defects than did single mutants, particularly in the left ventricle (Fig. 42.6). These

results indicate that the dosage of epigenetic factors affects the severity of the *Tbx5* mutant phenotype (i.e., left ventricular hypoplasia). The severity of congenital heart disease in humans may be related to the level of expression of epigenetic factors and/or of partner factors. To address this possibility, we need to elucidate the relationship between the expression level of epigenetic factors and the penetration of heart failure.

42.3 Future Directions and Clinical Implications

We have analyzed the functions of Baf60c, a component of the SWI/SNF-type chromatin remodeling complex, in heart development in vivo and in vitro, but the mechanism by which Baf60c is regulated is still unknown. An important question is whether the functions of Brg1 or Baf60c are altered in each type of tissue. If their functions do not vary, they may be regulated in a partner-dependent manner. One approach to confirm this hypothesis is to use ChIP-sequencing to compare Baf60c and Brg1 target genes in different tissues. Another question that must be addressed is how Baf60c's expression pattern and dosage are regulated and which molecule (s) participate in this regulation. We found a candidate transcription factor that directly regulates Baf60c expression, but this molecule alone could not explain the dynamic change in Baf60c's expression pattern. Further analysis is required to determine the molecular mechanisms of Baf60c regulation.

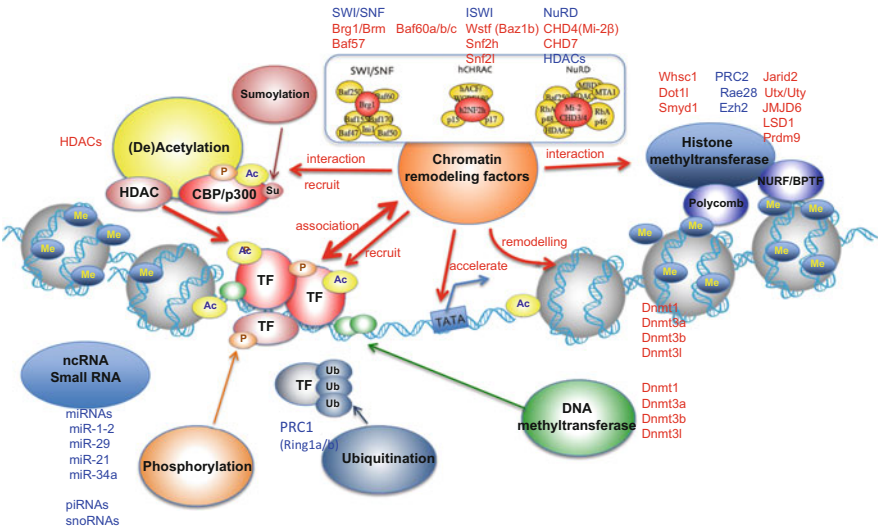

Fig. 42.7 Immunoprecipitation experiments indicate that Brg1 can strongly bind to Tbx5 with Baf60c. The lower panel shows the morphology of *Brg1del*/+, *Tbx5del*/+, and double heterozygote mouse hearts. The heart of the double heterozygote shows severe hypoplasia of the *left* ventricle (Adapted from Takeuchi et al. [20])

Over the last 20 years, cardiac researchers have elucidated many causes of congenital heart disease and have identified many genes that are involved. However, it is not sufficient to only understand the diversity or severity of the disease. In the future, we must also determine the role of epigenetic factors in heart failure because these factors regulate cardiac gene transcription (Fig. 42.7).

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