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The advances that have been made in understanding the developmental processes and molecular pathways that control cardiac development have been enormous in the last several decades. It was the original goal of the first Takao Symposia in 1978 to promote the scientific understanding of cardiac morphogenesis to form a foundation for investigating the mechanisms of congenital heart disease. However, even the ambitious investigators who attended that first meeting would not have anticipated the rapid progress that has occurred in this field. Furthermore, few would have predicted that there would be serious discussion about exploiting developmental paradigms to promote cardiac stem cell differentiation as an approach to attain cardiac regeneration. In this section we get a glimpse of some exciting new developments in these areas that have been the direct result of insights presented at and encouraged by the Takao Symposia.

One of the major advances in understanding the early stages of cardiac development has been the discovery that the entire heart does not arise from a single developmental field but rather the right ventricle, outflow track, and portions of the atria were derived from a second population of cells, now known as the second heart field (SHF), and the left ventricle appears to be primarily derived from anterior or the first heart field (FHF) derivatives. In this volume, Naremsu and Nakajima (Chap. 59) show that these distinct heart fields demonstrate differential response to the mutagen retinoic acid as early exposure of the FHF result in Transposition of the Great Vessels (TGA) and the double outlet right ventricle while exposure of the SHF at the same age results in persistence of the truncus arteriosus (PTA). Robert Kelley, one of the

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initial investigators to identify and delineate the SHF, and colleagues (Chap. 49) utilized an intricate combination of immunohistochemistry, Cre lineage tracing, transcriptome analysis, and novel *in vivo* morphological analysis to identify the heterogeneous nature of the SHF cell populations. They specifically detailed the epithelial like characterization of cell populations important in defining the transitional cell populations at both the arterial and venous poles of the SHF that are critical for normal morphogenesis. Kodo et al. (Chap. 58) added to the characterization of this heterogeneous SHF population by demonstrating that expression of the neurovascular molecule, semaphorin 3c, within the arterial pole of the SHF is essential for correct navigation of the cardiac neural crest cell (cNCC) populations essential for OFT development. Watanabe and Nakagawa (Chap. 60) expand the studies on novel factors regulating SHF and NCC interaction by showing that, *Hey1*, a downstream target of Notch 1 and *Alk1* signaling is critical in aortic arch formation as null animals display an increased incidence of right sided aortic arch, interrupted aortic arch, aberrant origin of the right sub-clavian artery. Further adding to the complexity of understanding the heterogeneity of SHF progenitor cell components, Kokubo et al. (Chap. 50) used a new lineage tracing model based on expression of secreted frizzled-related protein 5 (*Sfrp5*) to identify a novel progenitor cell population that contributes to the OFT, sinus venosus, left atria, and also to the left ventricle, which was previously thought to be primary, if not exclusively, derived from FHF progenitors. These studies raise the intriguing possibility of a single progenitor cell population that contributes to both the SHF and FHF. Regional heterogeneity of cardiac gene expression in distinct regions of the heart may not only be important for developmental regulation but may also play an important role in pathology of the mature organism. In a fascinating study, Steimle et al. (Chap. 51) used transcriptional profiling to examine genetic differences that may actually regulate early post-natal cardiac function as well as cardiac function in the adult. They were able to identify discrete differences in gene expression between the left atrial appendage and the peri-pulmonary vein region of the left atria which is believed to be the nidus for many forms of atrial fibrillation.

With the development of induced pluripotent stem (iPS) cell technology, scientific interest in factors that regulate cardiac stem cell differentiation into functional components of the mature heart has expanded well beyond the focus on early events of cardiac ontogeny as these cells could provide a foundation for future cell based regenerative therapies. New methods to identify differentiated progenitor cells *in vitro* will be required for clinical utilization of iPS cells. Kawaguchi et al. (Chap. 61) showed that c-kit, a stem cell factor receptor often used for identifying cardiac progenitor cell populations, demonstrated robust expression in iPS cells grown on feeder cells but was subsequently downregulated following mesodermal and myocardial differentiation. They suggested that this factor might affect the early stages of cardiomyocyte differentiation. In an elegant study, Morita and Takeuchi (Chap. 57) developed transgenic iPSCs that could be induced to differentiation in functional cardiomyocytes, without the addition of extrinsic growth factors, by induction of two critical factors that they identified in a single cell screening of early cardiac progenitors. While iPS cells can be differentiated into cells that express myocardial markers, a major limitation has been achieving myocyte maturation that

would achieve clinical utility. As such Brockmeier and co-workers (Chap. 55) present a novel *in vitro* model to quantify myocardial contractility of both embryonic stem cell (ESC) and iPS derived cardiomyocytes by assessing the ability of these cells to integrate, *in situ*, with both normal and damaged myocardial tissue slices instrumented for measurement of isometric force transduction. This system allows for precise pharmacologic manipulation and they found that ESCs and IPS cells showed quantifiable increases in force/contractility as measured in the system and few differences were noted between the two cell types. However, neither generates contractile forces comparable to native heart tissue and their work points to development of the sarcoplasmic reticulum as a limiting feature of ESC and iPSC differentiation strategies. In a “tour de force”, Keller and colleagues (Chap. 54) go beyond an isolated single cell iPSC strategy in favor of the use of multiple cell lineages in 3D matrix formulations to form implantable engineered cardiac tissues (ECTs) from several sources including human iPS cells. They showed that these ECT tissues had improved maturation, could be optically paced, and were capable of improving the myocardial function after infarction. They describe several recent modifications that enhance the potential use in human tissue engineering strategies. To further expand the utility of iPSCs, Hayama et al. (Chap. 62) were able to determine that iPSCs could be derived from immortalized B cell lines and successfully differentiated into cardiomyocytes. This dramatically expands the potential of the iPS methodology to evaluate human diseases as these cell lines have been utilized to identify genetic mutations associated with an array of CHDs and thus now provide a new source for *in vitro* disease modeling. The utility of iPSCs in modeling human diseases is further illustrated by the work of Furutani et al. (Chap. 63) who developed an *in vitro* model to study Long QT Syndrome 3 by generating iPSCs from patient derived immortalized B-cell lines.

While significant attention has been paid to the early stages of cardiac morphogenesis including organization of the first and second heart fields as discussed above, heart tube looping, septation, and valve formation during latter stages of cardiac morphogenesis have been inaccessible to laboratory investigation because of the early embryonic demise of many genetic mutants. However, newer Cre mediated deletion strategies allowing temporal and tissue specific deletion of genes have been increasingly utilized to interrogate gene regulation in later gestational events. Qu and Baldwin (Chap. 52) offer novel insights into the important dynamic process of ventricular trabeculation. Using Cre mediated endocardial specific deletion of the receptor tyrosine kinase Tie2, they showed that signaling via this RTK is required for endocardial proliferation and development of the endocardial “sprouts” that define the trabeculae. Perhaps equally important, they showed that endothelial Tie2 mediated paracrine signaling suppresses myocardial proliferation, thus regulating the proper number of myocytes in the developing ventricle. Ishida et al. (Chap. 56) showed that following trabeculation, Glial cell line-derived neurotrophic factor receptor alpha (*Gfra*) is required in subsequent stages of ventricular compaction as *Gfra1&2* compound mutants developed a no-compaction cardiomyopathy. Furthermore, this phenotype was the result of perturbation in non-canonical Notch signaling. Nogimori et al. (Chap. 64) turned to human genetic studies to document

the clinical variability in the process of left ventricular non-compaction by comparing one case of Gata4 mutation which was only mildly symptomatic with a patient harboring a TBX20 mutation who showed severe pulmonary hypertension and diastolic dysfunction. In a fascinating study, Steimle et al. (Chap. 51) used transcriptional profiling to examine genetic differences that may actually regulate post-natal cardiac function after birth and in the adult. They were able to identify discrete differences in gene expression between the left atrial appendage and the peripulmonary vein region of the left atria which is believed to be the nidus for many forms of atrial fibrillation.

The advances that have occurred since the first Takao Symposia have truly been impressive. Yet as Nakagama and Inuzuka remind us (Chap. 65) there are still significant challenges that remain in elucidating the pathogenesis of CHD and particularly challenging is developing vertebrate models that recapitulate human CHD to distinguish truly causal genetic variants from benign mutants. In addition, Dr. Bittel (Chap. 53) elegantly describes a potential role of non-coding RNAs in the etiology of Tetralogy of Falot further complicating the possible genetic mechanisms leading to CHD. It is clear that the mechanisms of normal and abnormal cardiac development are much more complicated than ever imagined. But based on the progress seen since the first Takao Symposia, we can be optimistic that the mechanisms and treatments for CHD will ultimately be unraveled.

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