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Mini Review

Current Update on Genomics of Cardiac Embryogenesis

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Abstract

Genetic pathways that control cardiac embryogenesis follows intricate temporal patterns. Concept of first and second heart fields, a population of potent cardiac cells that initiate the simple heart tube, has been deciphered. Role of genes and various controlling factors in multiplex regulatory systems that drive organogenesis that is altered in disease needs to recognised and is one of the greatest challenges in science. Understanding and control of these steps may lead to a revolution in auto engineered artificial organs and targeted therapy of congenital heart disease and early detection of these lesions.

Keywords: *Cardiogenesis; Gene Regulatory Network; Id Proteins; Kernel Factors; Micro Rna; Neural Crest; Transcription*

Early cardiogenic genes

In Caenorhabditis elegans, the rhythmically contracting pharynx is the most primitive form of heart field [1]. Cephalochordate Amphioxus has a contractile vessel [2]. Cardiac transcription factors NK2 Homeobox 5 (NKX2.5) or Myocyte Enhancer Factor 2 (MEF2) homologues find expression in muscles of pharynx of these nematodes.

During cardiac embryogenesis in Drosophila or humans transcription factors NKX2, MEF2, GATA binding protein (GATA), T-box and neural crest derivatives are involved [1]. These were first identified in Drosophila before their role in vertebrate heart development was noted [3]. Family of T-box transcription factors play a key role in the regulation of cardiomyocyte identity. Uniqueness of expression determines the electrical pattern of heart. Most cardiac regions express TBX5 and TBX20. TBX5 is expression inclined from the venous pole to the right ventricle with a notable absence in the region of outflow tract. Both are important activators of gene expression in the chamber development. TBX2 and TBX3 act as repressors of gene programming of the inflow tract, or venous pole, atrioventricular canal and outflow tract regions – leading to another developmental destiny. Outflow tract precursors of the heart, TBX1 is expressed and TBX18 are expressed at the venous poles. When expression of these genes are affected congenital heart defects result, showing their importance in cardiac development [4]. The identification of the first Drosophila cardiogenic gene Tinman (TIN) [3,5,6]. has led to the cloning of the mouse NKX2-5 gene [7,8]. Transcription factors and signalling pathways involved in invertebrate cardio genesis, are needed for vertebrate heart development with similarities in the gene regulatory network (GRN) [1,9]. The early polarity expression of SERCA2 (calcium

pump of the endoplasmic reticulum) and PLB (phospholamban) is unique as polarity is expressed before commencement of contractions. SERCA2 concentration decreases from anterior to posterior regions, while PLB shows complementary distribution [10,11]. In Xenopus, the expression of NKX2.5 is restricted to the internal lateral plate of the precardiac crests [12].

Clear regionalization of expression patterns are noted in transcription factor PITX2[13,14]. PITX2 is expressed in the left precardiac crest, but not in the right, being configured as the first sign of molecular asymmetry in cardiac embryogenesis. Homeo box factor- IRX4, expression is restricted to the anterior region of the crest [15]. Cells that express IRX4 constitute the ventricular primordium. Homogenous expression of transcription factors are also noted in precardiac crests- TBX 5, SRF (serum response factor), CARP (Cardiac Ankyrin Repeat Protein), pCMF1, Midori, c CLP 1 and MESP1 [16-21].

The Drosophila heart development depends on a regulatory network of TFs homologous to the core factors in mammals, NKX2-5-like homeo domain factor Tinman, GATA factor Pannier and T-box factor Dorsocross [22]. TFs, MEIS1 and MEIS2 interact with HOX and PBX TFs in cardiac embryogenesis [23,24]. Sequencing of human genome has given the much necessary thrust. Uniqueness of transcription rather than the number of protein-coding genes as the evolutionary hall mark for biological diversity is the recent addition of technological advances in this field [26-28]. Selector genes are the master regulators e.g. muscle-specific TF MyoD1, which induces muscle fate on expression induction [29]. Early in embryogenesis allocation of cardiac parentage within a common mesodermal field is subject to antagonistic principles with genetic disruption of transcription factors (TFs) which determines domination pattern of development [30-32]. Hall mark of mammalian cardio genesis is the septation of a single tubular form to create four chambers with different roles and identities: two atria and two ventricles with flow direction provided by atrioventricular valves [33]. In mice, the heart tube begins to develop from a crescent-shaped population of progenitors composed of two distinct fields of cells with different behavioural pattern and cell lineage fates: the first and second heart fields -FHF and SHF [34].The FHF originates from the anterior splanchnopleuric mesoderm and gives rise to the linear heart tube composed of left ventricle. The SHF is derived from branchial mesoderm situated medial to the cardiac crescent and comes to lie dorsally. This population becomes extensive by proliferation, and populates the growing heart at its inflow and outflow tracts contributing to the formation of atria, right ventricle, inflow and outflow tract. In mice, cranio-caudally heart is formed by convergence of the FHF progenitors at the midline at around E8.0 and begins to beat owing to the automaticity of myocytes [34]. Linear heart tube begins elongating around E8.5, as a result of deployment of SHF cells, it undergoes a spiral looping towards right under the influence of the left–right asymmetry genetic network [35]. Precursor cells derived from the neural crest, proepicardial regions and sinus venosus also migrate and integrate into the heart, collectively contributing to formation of cardiac ganglia, smooth muscle, and endothelial cells of the coronary vessels, and fibroblasts of the interstitial and annulus fibrosus. A number of deeply conserved mammalian cardiac TFs for target genes, can be considered to be part of a cardiac kernel including GATA4/6, ISL1, NKX2-5, MEF2C, SRF, TBX5, and TBX20. Knockout of kernel factors leads to arrested heart development, with phenotypes showing unique features [36-38]. Combinations of cardiac TFs centred on GATA4, TBX5, and MEF2C, with or without the inclusion of specific micro-RNAs (mi RNAs) or small molecules modulating signalling and epigenetic states, reprogram non cardiac embryonic or adult cells to a cardiomyocytes [39,40]. Alternative method of evaluating cardiac function in adult flies was by optical coherence tomography (OCT), which was developed in the laboratory of Wolf and Rockman [41]. Using OCT [42] screened for molecularly defined deficiencies in the Drosophila chromosome 3Lcan be done. Anterior embryonic and extra embryonic mesoderm, MESP1 is expressed from gastrulation and is capable of stimulating cardio genesis [43,44]. Gatekeeper of pluripotency - homeodomain factor, OCT4 is involved in early lineage and is co expressed with MESP1 during mouse pluripotent stem cell differentiation into cardiac lineages. Here the regulation is dose dependent [45,46]. RNA i-based genome-scale with loss of function screen has been described [47]. Taking advantage of a β-galactosidase cardiac-specific expression of MEF2, more than 5800 genes have been screened for heart mutant phenotypes by injection of double-strand RNA (dsRNA) in the developing embryo. 132 genes have been identified so far, including genes encoding TFs and cell signalling molecules, involved in different steps of cardiac development. For mutants presenting heart defects, genetic screens were conducted in transgenic lines bearing a green fluorescent protein (GFP), allows examination of mutant phenotypes in live embryos.

At transcriptional and post-transcriptional levels, the development of the heart is regulated. Specific regulation at the post-transcriptional level involves small non-coding RNA molecules called microRNAs (miRs). These regulate gene expression by binding to specific sequences within the messenger RNAs and reducing their stability from being translated into proteins. Precise miR activity is required for the heart to develop normally and able to respond to challenges such as ischemia and pressure overload. Cardiac enriched miRs which are currently known are miR-1, 133, 206, 208 and 499 [49], with miR-1 being the most abundant in the adult mouse heart.

MiR-1 is co-transcribed with miR-133a, and has two copies in the mouse genome, miR-1-1 on chromosome 2 and miR-1-2 on chromosome 18 [50], Id proteins specify cardiac cell fate by repressing two inhibitors of cardiogenic mesoderm formation - Tcf3 and Foxa2 - and activating inducers Evx1, Grrp1, and MESP1. CRISPR/Cas9-mediated ablation of the entire Id (Id1- 4) family in mouse embryos leads to failure of anterior cardiac progenitor specification and the development of heartless embryos [51],

Cardiac jelly from the neural crest triggers directional signals between endocardium and myocardium. The left right asymmetry with a loop rightward is due to sense directional flow of extra embryonic coelomic fluid generated by ciliary cells [52]. BMP2/4 expression determines the early cardiogenic crescent formation by NKX2-5 TFs which are expressed in mesenchymal cells [53]. In protein coding genes that are close to lnc RNAs, Hori Y., *et al*. [54] found many transcription factor genes that have critical functions for heart development (i.e., TBX5, TBX20, NKX2–5, GATA4, GATA6, SALL4, HAND1, HAND2, Wt1, Nr2f1, IRX3 and IRX5). Many of these lnc RNAs were bidirectional lnc RNAs (i.e., TBX5, TBX20, NKX2–5, Gata6, SALL4, HAND1, HAND2, Wt1, Nr2f1, IRX3 and IRX5).

Conclusion

Impact of single or multiple gene variants needs precise delineation if the knowledge is to be applied as a clinical tool, therapeutic armamentarium or for genetic engineering. Many foetal tissues reactivate programs of gene expression when damaged, revealing the potency inherent in genetic programming. This potential ought to be exploited in regenerative medicine and other genomic therapeutic options. Deciphering the spatial and temporal targeted gene expression or gene inactivation could be the lead areas of genomic diagnostic and therapeutic interventions of future.

Bibliography

1. Olson EN. “Gene regulatory networks in the evolution and development of the heart”. *Science* 313. 5795 (2006): 1922-1997.
2. Moorman AF and Christoffels VM. “Cardiac chamber formation: development, genes, and evolution”. Physiological Reviews 83 (2003): 1223-1267.
3. Bodmer R. “The gene tinman is required for specification of the heart and visceral muscles in Drosophila”. *Development* 118.3 (1993): 719-729.
4. Boogerd CJJ., “Protein interactions at the heart of cardiac chamber formation”. *Annals of Anatomy* 191 (2009): 505-517.
5. Azpiazu N and Frasch M. tinman and bagpipe: two homeo box genes that determine cell fates in the dorsal mesoderm of Drosophila. *Genes & Development* 7(7B)(1993): 1325-1340.
6. Bodmer R and Frasch M. “5 - Genetic determination of Drosophila heart development”. In: Rosenthal N, Harvey RP (eds). Heart Development. San Diego, California: Academic Press, (1999): 65-90.
7. Komuro I and Izumo S. “Csx: a murine homeobox-containing gene specifically expressed in the developing heart”. *Proceedings of the National Academy of Sciences* 90.17 (1993): 8145-8149.

8. Lints TJ., *et al*. "NKX-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants". *Development* 119.3 (1993): 969.

9. Cripps RM and Olson EN. "Control of cardiac development by an evolutionarily conserved transcriptional network". *Developmental Biology* 246.1 (2002): 14-28.

10. Moorman AFM., *et al*. "Patterns of expression of sarcoplasmic reticulum Ca2+ATPase and phospholamban mARNs during rat heart development". *Circulation Research* 76 (1995): 616-25 .

11. Moorman AFM., *et al*. "Presence of functional sarcoplasmic reticulum in the developing heart and its confinement to chamber myocardium". *Developmental Biology* 223 (2000): 279-290.

12. Raffin M., *et al*. "Subdivision of the cardiac NKX25 expression doamin into myogenic and nonmyogenic compartments". *Developmental Biology* 218 (2000): 326-340.

13. Piedra ME., *et al*. "PITX2 participates in the late phase of the pathway controlling left-right asymmetry". *Cell* 94 (1998): 319-324.

14. Campione M., *et al*. "PITX-2 expression defines a left cardiac lineage and provides evidence for the existence of ventricular isomerism in mice". *Developmental Biology* 231 (2001): 252-269.

15. Bruneau BG., *et al*. "Cardiac expression of the ventricle-specific homeobox gene IRX4 is modulated by NKX25 and d HAND". *Developmental Biology* 217 (2000): 266-277.

16. Croissant JD., *et al*. "Avian serum response factor expression restricted primarily to muscle cell lineages is required for α-actin gene transcription". *Developmental Biology* 177 (1996): 250-264.

17. Saga Y., *et al*. "MESP1: A novel basic helix-loop-helix protein expressed in the nascent mesodermal cells during mouse grastrulation". *Development* 122 (1996): 2769-2778.

18. Zou Y., *et al*. "CARP, a cardiac ankyrin repeat protein, is downstream in the NKX25 homoebox gene pathway". *Development* 124 (1997): 793-804.

19. Ghatpande S., *et al*. "Identification of a novel cardiac lineage-associated protein (cCLP-1): A candidate regulator of cardiogenesis". *Developmental Biology* 208 (1999): 210-221.

20. Wei Y., *et al*. "Identification of a novel cardiac-specific transcript critical for cardiac myocyte differentiation". *Development* 122 (1996): 2779-2789 .

21. Hosoda T., *et al*. "A novel myocyte-specific gene Midori promotes differentiation of P19CL6 cells intro cardiomyocytes". *The Journal of Biological Chemistry* 276 (2001): 35978-35989.

22. Jin H., *et al*. "Genome-wide screens for in vivo Tinman binding sites identify cardiac enhancers with diverse functional architectures". *PLOS Genetics* 9 (2013): e1003195.

23. Paige SL., *et al*. "A temporal chromatin signature in human embryonic stem cells identifies regulators of cardiac development". *Cell* 151 (2012): 221-232.

24. Wamstad JA., *et al*. "Dynamic and coordinated epigenetic regulation of developmental transitions in the cardiac lineage". *Cell* 151 (2012): 206-220.

25. Lander ES., *et al*. "Initial sequencing and analysis of the human genome". *Nature* 409 (2001): 860-921.

26. Britten RJ and Davidson EH. "Gene regulation for higher cells: A theory". *Science* 165 (1969): 349-357.

27. Carninci P., *et al*. "The transcriptional landscape of the mammalian genome". *Science* 309 (2005): 1559-1563.

28. Fantom Consortium. "A promoter-level mammalian expression atlas". *Nature* 507 (2014): 462-470.

29. Tapscott SJ., *et al*. "MyoD1: A nuclear phosphoprotein requiring a Myc homology region to convert fibroblasts to myoblasts". *Science* 242 (1988): 405-411.

30. Schoenebeck JJ., *et al*. "Vessel and blood specification override cardiac potential in anterior mesoderm". *Development Cell* 13 (2007): 254-267.

31. Simoes FC., *et al*. "Fgf differentially controls cross-antagonism between cardiac and haemangioblast regulators". *Development* 138 (2011): 3235-3245

32. Van HANDel B., *et al*. "Scl represses cardiomyogenesis in prospective hemogenic endothelium and endocardium". *Cell* 150 (2012): 590-605.

33. Anderson RH., *et al*. "The development of septation in the four-chambered heart". *The Anatomical Record (Hoboken)* (2014).

34. Vincent SD and Buckingham ME. "How to make a heart: The origin and regulation of cardiac progenitor cells". *Current Topics in Developmental Biology* 90 (2010): 1-41.

35. Vandenberg LN and Levin M. "A unified model for left-right asymmetry? Comparison and synthesis of molecular models of embryonic laterality". *Developmental Biology* 379 (2013): 1-15.

36. Watt AJ., *et al*. "GATA4 is essential for formation of the proepicardium and regulates cardiogenesis". *Proceedings of the National Academy of Sciences* 101 (2004): 12573-12578.

37. Stennard FA., *et al*. "Murine T-box transcription factor TBX20 acts as a repressor during heart development, and is essential for adult heart integrity, function and adaptation". *Development* 132 (2005): 2451-2462.

38. Prall OWJ., *et al*. "An NKX2-5/Bmp2/Smad1 negative feedback loop controls heart progenitor specification and proliferation". *Cell* 128 (2007): 947-959.

39. Ieda M., *et al*. "Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors". *Cell* 142 (2010): 375-386.

40. Chen JX., *et al*. "Somatic cell reprogramming into cardiovascular lineages". *Journal of Cardiovascular Pharmacology* (2014).

41. Wolf MJ., *et al*. "Drosophila as a model for the identification of genes causing adult human heart disease". Proceedings of the National Academy of Sciences USA 103.5 (2006): 1394-1399.

42. Yu L., *et al*. "Affecting Rhomboid-3 function causes a dilated heart in adult Drosophila". *PLOS Genetics* 6.5 (2010): e1000969.

43. Bondue A., *et al*. "MESP1 acts as a master regulator of multipotent cardiovascular progenitor specification". *Cell Stem Cell* 3 (2008): 69-84.

44. Bondue A., *et al*. "Defining the earliest step of cardiovascular progenitor specification during embryonic stem cell differentiation". *The Journal of Cell Biology* 192 (2011): 751-765.

45. Zeineddine D., *et al*. "Oct-3/4 dose dependently regulates specification of embryonic stem cells toward a cardiac lineage and early heart development". *Dev Cell* 11 (2006): 535-546.

46. Blin G., *et al*. "A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates". *Journal of Clinical Investigation* 120 (2010): 1125-1139.

47. Kim YO., *et al*. "A functional genomic screen for cardiogenic genes using RNA interference in developing Drosophila embryos". Proceedings of the National Academy of Sciences USA 101.1 (2004): 159-164.

48. Tao Y., *et al*. "Second chromosome genes required for heart development in Drosophila melanogaster". *Genesis* 45.10 (2007): 607-617.

49. J Chen and DZ Wang. "microRNAs in cardiovascular development". *Journal of Molecular and Cellular Cardiology* 52 (2012): 949-957.

50. Y Zhao., *et al*. "Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2". *Cell* 129 (2007): 303-317.

51. Thomas J., et al. "Id genes are essential for early heart formation". *Genes Development* 31.13 (2017): 1325-1338.

52. Pradeep Kumar Radhakrishnan., *et al*. Deciphering Cardiac Embryogenesis in Congential Heart Disease - The First Step Towards an Autoengineered Heart?. *Frontiers in Paediatric Medicine* (2019).

53. Harvey RP., *et al*. "Homeodomain factor NKX2-5 in heart development and disease". *Cold Spring Harbor Perspectives in Medicine* 67 (2002): 107-114.

54. Hori Y., *et al*. "Important cardiac transcription factor genes are accompanied by bidirectional long non-coding RNAs". *BMC Genomics* 19. 967 (2018).

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