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Super Enhancers: Enhancing Human Cardiogenesis

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Congenital heart defects remain one of the most common developmental defects within the global human population, affecting up to 1% of all live births, and with increasing prevalence[1]. The abundance of these cardiac defects attests to the complexity of the developmental process, which requires specific and precise spatiotemporal control of gene expression. Early models focused on transcription factors; however, comparisons between mouse and human genomes revealed that less than half of conserved sequences encoded proteins, suggesting that the noncoding regions of DNA were just as important for development as the coding regions[2]. Interestingly, early genome-wide association studies (GWAS) also found significant susceptibility to disease mapping to noncoding regions[3], supporting the notion that elements beyond transcription factors must aid in not only development but also disease susceptibility. Furthermore, these sequences were found to be tightly restrained to specific developmental time points[4] and specific cell types, reinforcing them as key components of the gene regulatory system. Recent studies have identified noncoding sequences that regulate gene transcription, including enhancers, and provide an additional level of hierarchical gene regulation[5].

Enhancers function as a scaffold for transcription machinery. Acetylation of histone H3 lysine 27 (H3K27ac) and mono-methylation of histone H3 lysine 4 (H3K4me) within enhancers facilitate recruitment of transcription factors to initiate macromolecular complex formation, the enhanceosome. The mechanism by which the enhanceosome forms is incompletely understood but appears to follow one of two models: instructive or permissive. In the instructive model the regulatory structure is formed *de novo* in specific cell types, while in the permissive model, the structure is pre-formed but only activated in specific cell

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types[6]. Once the enhanceosome is assembled, it is able to increase the activity of RNA polymerase II seemingly via alignment of the transcription machinery in space[7]. Due to their geometry and function as spatial regulators, enhancers are not limited to close proximity to their target gene but can function from thousands, or even millions, of bases away[8]. Unlike transcription factors, enhanceosomes do not function like an on/off switch, but rather titrate the level of the transcription[9, 10].

The importance of these regulatory sequences is further supported by recent studies which found significant redundancy within the enhancer program during development[5, 11]. The redundant enhancers appear to function in an additive manner regarding strength of the target gene transcription level, suggesting a robustness within the program[11]. Within the last decade, multiple studies, including ENCODE and Roadmap Epigenome projects, have identified enhancers through characterization of DNA accessibility and various histone modifications in developing vertebrate systems[2, 12, 13]. Recent studies have also demonstrated strong association between congenital cardiovascular disease and mutations within enhancer regions[14, 15]. However, comprehensive identification of active enhancers at various stages of heart development – particularly in humans – is lacking.

In the current issue of *Circulation Research*, a study by VanOudenhove *et al*[16] begins to fill this gap in our understanding by identifying and characterizing cardiac-specific active enhancers at various stages of human heart development. In their work, VanOudenhove *et al* utilized ChIP-seq against seven histone H3 post-translational modifications across Carnegie stage 13, 16 and 23 human embryonic hearts. By comparing the non-coding enhancer segments across the time points and analogous annotations from all tissues in Roadmap Epigenome, authors identified over 9,000 novel putative embryonic heart-specific enhancers that are differentially active during cardiogenesis. Active enhancers at early stages of cardiac development revealed enrichment of SOX2, OCT4, KLF, and FOX transcription factor binding motifs. During later stages of cardiogenesis, active enhancers exhibited a pronounced shift in enrichment, mostly towards T-box, GATA, PAX, and Zinc Finger transcription factor binding motifs. Interestingly, several enhancer regions exhibit robust active state throughout embryonic cardiogenesis but repressed state in fetal and adult human hearts, suggesting coordinate activation of super-enhancers during early cardiac development. These ~1600 embryonic human heart specific super-enhancers are often located near critical cardiogenesis genes, including *NKX2–5*, *SCN5A*, *HAND2*, *TBX20*, *GJA1*, and *MYOCD*, frequently mutated in patients with congenital heart anomalies.

By comparing embryonic human heart specific super-enhancers against a GWAS catalogue of congenital heart defects, the authors identified significant enrichment of variants associated with atrial fibrillation. While enhancers have been associated with atrial fibrillation in the past[17], the work discussed here is unique in that it identifies activation of these enhancers during the earliest stages of cardiac development. Such findings suggest that atrial fibrillation may be a congenital cardiac disease – an enhanceropathy – rather than an acquired disease. The work thus begs the question of what other cardiac conditions, commonly felt to be acquired, may be primed in the earliest days of cardiac development. A second possibility is that atrial fibrillation triggers a reversion to fetal gene program, which would have important implications for future therapeutic development.

Rather than relying on the identification of specific genes alone, the work goes one step further and examines the roles of networks of gene activation. This approach appears to be more representative of the biology of disease and development, and potentially more clinically relevant. In this study, VanOudenhove *et al* hypothesized that "hub" genes, which are located at the interface of several cardiac development pathways, could be unidentified regulators of congenital heart defects given their ability to affect multiple genetic programs. To test this hypothesis, the group utilized a weighted gene co-expression network analysis using all of their profiled embryonic human heart samples and investigated modules associated with early heart development and loss-of-function of variants. Through this method, they identified over 250 genes that appear to play a pivotal role in cardiac development with a low tolerance for gene disruption, suggesting they may function as potential diagnostic or therapeutic targets for developmental cardiac defects.

The findings by VanOudenhove *et al* suggest that the answers to many of our questions regarding congenital heart disease and even acquired disease, may be hiding in plain sight in the form of embryonic super-enhancers. While not the typical "on/off switch" of the classic transcription factors, enhancers fine tune gene expression and enhanceropathies are a testament to the sensitivity of the biological system to protein expression level[9, 10]. Such results are relevant to not only congenital disease, but also acquired disease as recent work has demonstrated enhancer activation from environmental signals[18], potentially revealing an entirely new target for future treatments of cardiovascular disease.

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