Epigenetics and Congenital Heart Diseases

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Congenital heart disease (CHD) is a frequent occurrence, with a prevalence rate of almost 1% in the general population. However, the pathophysiology of the anomalous heart development is still unclear in most patients screened. A definitive genetic origin, be it single-point mutation or larger chromosomal disruptions, only explains about 35% of identified cases. The precisely choreographed embryology of the heart relies on timed activation of developmental molecular cascades, spatially and temporally regulated through epigenetic regulation: chromatin conformation, DNA priming through methylation patterns, and spatial accessibility to transcription factors. This multi-level regulatory network is eminently susceptible to outside disruption, resulting in faulty cardiac development. Similarly, the heart is unique in its dynamic development: growth is intrinsically related to mechanical stimulation, and disruption of the intrauterine environment will have a direct impact on fetal embryology. These two converging axes offer new areas of research to characterize the cardiac epigenetic regulation and identify points of fragility in order to counteract its teratogenic consequences.

Keywords: congenital heart disease ; epigenetics ; genetics

1. Introduction

Congenital heart disease (CHD)—structural defects in heart morphology—is one of the most-represented types of congenital anomalies, with a prevalence of 1% in the general population [1]. Despite the wide array of potential mistakes, the spectrum of observed phenotypes in patients intriguingly converge in a finite set of entities [2]. After multiple systems of patient classification, the most logical and medically appropriate emerged from heart embryology: the various phenotypes in CHD can be easily related to embryological errors, and precise knowledge of the sequential events and spatiotemporal interactions between heart substructures is intrinsic to understanding pathophysiology [3].

With the great genetic revolution came the hope that unlocking the molecular bases for CHD would provide fluid gene/disease correlations; this sadly was far from the case in the domain of congenital cardiac diseases. Today, with the advances in genome annotations and mass sequencing of patient DNA, only 35% of all occurrences can be clearly linked to a genetic origin, be it single-gene mutations (3–5%), aneuploidies (8–10%), or copy number variants (3–25%) [4]. Even in the case of identified genetic substratum, the mutation/disease model remains inapplicable, as is evidenced by (1) the convergence of various mutations to identical phenotypes; (2) conversely, the multiplicity of phenotypes associated with a single identified mutation; and (3) the incomplete penetrance of these mutations [5]. The key to understanding the anatomical spectrum of CHD therefore lies in epigenetics, a concept here encompassing all modifiers external to the genetic code in itself, affecting the temporality and level of expression of these genes as well as modifying environmental factors.

Epigenetic regulation is multidimensional in nature, and plays a crucial role in heart organogenesis, as evidenced by the high prevalence of CHD in syndromes involving epigenetic regulator mutations: histone-modifying genes alone are estimated to contribute to 10% of CHD patients [6]. Ergo, disruption of these regulatory mechanisms offers a convincing mechanism for explaining CHD prevalence and polymorph presentation within an identified genetic background, as suggested by the numerous examples of divergent monozygotic twins [7][8]. Intrinsically, heart embryology is shaped by interactions with its environment, and particularly with hemodynamic signalization, making it both specifically vulnerable to aberrant development in response to external hemodynamic stimulus; but also conferring it a high degree of adaptability, hereby explaining the finite spectrum of phenotypes encountered as viable up to birth. The highly complex and multiple regulatory levels in genetic regulation make it eminently difficult to unravel the precise mechanism in which epigenetic regulators—maternal environment, hemodynamic variations, micronutrient availability, toxicant exposure—mediate embryological errors. However, as data accumulate, these factors could bridge the gap between identified and unexplained cardiac pathogenesis and allow for potential protective intervention.

2. Morphogenesis, Embryology and Disease Spectrum

Morphogenesis of the heart is established very early on in organogenesis, between 15 and 45 days after conception. The emergence of a functional circulatory system is crucial to the survival of the embryo, as tissular diffusion alone becomes unable to cope with the rapidly increasing size of the embryo [9]. At day 30, most major cardiac structures have been established in their spatial conformation; further remodeling will occur later on at a smaller scale in tissue anatomy, with the development of the supporting vasculature and the maturation of heart trabeculations being the closing points on organ development [10].

Heart progenitors can be identified from day 15 onwards, localizing in a crescent-shaped region known as the first heart field (FHF) [11]. Medially to this heart crescent, a second area, the Second Heart Field (SHF), will also contribute to heart morphogenesis through a mechanism of addition, building throughout cardiogenesis upon the scaffold established by the FHF skeleton [12]. The FHF undergoes fusion at the midline, establishing a primitive linear cardiac tube. Spatial localization within the tube (and ergo the loop) is intrinsically linked with cellular fate and function: cellular identity and organ asymmetry are established in a two-dimensional axis in the first phase of development (left/right lateralization) within the crescent-shaped heart fields and fine-tuned in three-dimensional spatial regulation at the tube-formation phase [11]. Throughout the subsequent steps of cardiogenesis, proper development will be wholly reliant on effective crosstalk between the various cellular progenitors, including FHF, SHF and neural crest cells [13]. Errors in cellular migration, cellular interaction, regulated apoptosis and proliferation will affect the alignment of structures, tissular growth and the resulting flow pattern throughout the developing organ, resulting in the wide pathological spectrum of CHD [14][15][16].

A functional, beating heart is indispensable for embryogenesis to pursue, and anomalies affecting cardiac structure result in a dichotomous outcome: viability or spontaneous abortion. A high number of spontaneous miscarriages are thought to result from unviable cardiac phenotypes [17]. The genetic cascades activated throughout heart development are exclusively fundamental cellular pathways: cell proliferation and apoptosis, cell migration and embryo lateralization. High-impact point-mutation on such primitive signalization pathways would never result in viable pregnancies, explaining the low percentage of identified point-mutation in CHD cohorts, and seemingly implicates cardiac specific transcription factors in many cases: GATA4-6, NKX2.5, TBX1, NOTCH, JAG to cite a few that are frequently identified in genetic screening of patient cohorts [18]. It can be postulated that hits on these molecular “switches” could potentially be bypassed with parallel redundant cascades, allowing for potential “rescue”—hence explaining the variability of phenotypes stemming from identical point-mutations and once again introducing the concept of “modifiers”—i.e., epigenetics. Genome-wide analysis is now conceptualized as veritable networks of genes, each node influencing and regulating multiple adjacent factors [19]: the regulation of expression, more than the gene product itself, becomes a potential answer in understanding CHD.

3. Epigenetic Alterations in Heart Disease

In recent years, multiple levels of gene expression regulation have been discovered and explored; mutations on these regulatory elements understandably result in multisystemic involvement. Congenital heart defects are not spared from this, and both in vivo and patient observations have shed light on the major role epigenetics play in CHD.

Within the nucleus, excluding the tightly packed chromosomal state of mitosis, DNA resides as an unwound double-strand: the DNA molecule is arranged in specific conformations, within spatially defined domains. Within these domains, regulatory elements and multiprotein complexes act as scaffolds for bringing in contact distant genomic regions for combined temporospatial expression. These zones are known as topologically associated domains and allow for co-regulation of genetic targets [20]. Establishment of TAD is under the control of architectural proteins such as CTCF, which delimitates the borders of topological domains. Cohesin complexes also play a role in the dynamic function of TADs: this circular protein structure is loaded unto the DNA strand and will progressively extrude the chromatin into expanding loops until they run into insulator proteins (CTCF), bump into each other or dissociate from the molecule, effectively bridging together distant genomic regions [21]. Disruption of these regulatory mechanisms, as evidenced by many examples in pathophysiology, will directly result in CHD phenotypes. Cornelia de Lange syndrome, resulting from mutations within the cohesin complex (NIPBL, HDAC), frequently manifests with CHD, TOF in 50% of cases, VSD, ASD, PDA and valvular abnormalities [22].

Accessibility of transcriptional machinery to its target sites will depend on the local “openness” of the genetic information. The DNA filament is packed around protein units, an octamer of histones organizing the chromatin in functional units known as nucleosomes. The nucleosomes will directly regulate how tightly DNA is packed through electrostatic interaction with the adjacent histones and DNA itself. To effectively open and close chromatin, the histone tails are modified through acetylation, phosphorylation and deacetylation; these tags may play a permissive or repressive role in gene accessibility

[23]. Mutations in SMAD2, a regulator of H3 methylation, were shown to be excessively represented in Whole-Exome analyses comparing CHD versus unaffected subjects [6]. T-box proteins, mutated in Holt-Oram syndrome (TBX5) and DiGeorge syndrome (TBX1), display highly conserved residues, allowing direct interaction with both histone demethylase (H3K27) and methyltransferase (H3K4): both syndromes include cardiac manifestations [24], underlining the highly regulatory nature of these pathogenic mechanisms; treatment with demethylase inhibitors of Tbx1-KO mice rescues the cardiac phenotype [25]. Histone acetyltransferase variants were shown to be associated with CHD (ventricular septal defects, atrial septal defects, patent ductus arteriosus and tetralogy of Fallot) in a Chinese Han cohort [26], perhaps hinting at a mechanism for polygenic susceptibility models. Histone modifications also have the advantage of being highly dynamic in nature and allowing time-specific regulation of gene expression: PRDM6, a methyltransferase involved in maintaining cells in an undifferentiated stage with proliferative potential is highly expressed in ductal tissue, and will drastically fall in the postnatal phase, allowing for differentiation and ductus arteriosus closure. Disruption of its activity results premature differentiation and persistent ductus arteriosus [27].

Once the chromatin has been established as open and accessible, direct DNA methylation can once again orient transcription profiles by restricting or priming anchorage of transcription machinery. Addition or removal of methyl groups to nucleotide regions by methylases and demethylases is an extremely dynamic and fine-tuned way of adjusting the accessibility of genic domains by impeding attachment of transcription factors or gene-expression protein complexes. Multiple studies have proven differential methylation, both at the genome-wide level and specific coding regions, in CHD-affected patients [28][29][30]. Even more specifically, different methylation patterns are observed within discordant monozygotic twins for tetralogy of Fallot and the double outlet right ventricle—although the global genome-wide methylation burden does not differ, specific promoters are highly divergent in CpG marking, and can be linked back to cardiac embryology (TBX20, NFATC1 involved in valve formation, GATA4, NKX2-5, NOTCH4) [31][32]. Methylation plays a crucial role in embryology: cells undergo a widespread wave of demethylation to reestablish pluripotency during fertilization. As differentiation progresses, embryologic cascades seem to be progressively switched off through inhibiting methylation (hereby protecting the organisms from unregulated proliferation and cancerous predisposition) [33]. Conversely, targeted regions in specific cell precursors are actively kept in a demethylated state, in veritable cell-specific patterns, making them rapidly available for future activation. Knockout of demethylation enzyme TET1 in vivo recapitulates this mechanism, as affected cells display inhibitory hypermethylation at the NKX2-5 promoter, to cite one of many, effectively blocking cardiomyocyte differentiation [34]. This allows the future identity of yet-undifferentiated progenitors to be established at the very initial steps of heart development.

Finally, recruitment of multiprotein complexes interacting with DNA in all of these stages upstream of actual gene expression—methylation, histone modification, DNA conformation modification—can be in itself regulated by regulation molecules. Non-coding RNAs are emerging as prime candidates for this trans-acting modification: acting as scaffolds for machinery assembling and targeting, or sponges for dosage regulation of the transcribed RNAs, they add another intermediary step before protein expression which can be influenced by outside modifiers. Variations in levels of non-coding RNA have been explored in multiple studies and provide further support to their implication as regulators: targeted knockdown of cardiac-specific lncRNAs such as Handlr and Atcayos proved in vivo interaction with crucial cardiac nodes such as HAND2 and BMP4 [35]. In bicuspid aortic valve patients, miR-29 seems to be specifically downregulated [36]. However, as underlined by George et al., ncRNAs seem to have modest and easily bypassed impacts on cell fate, and so far have not been linked to CHD in large-scale screens [35].

Through this quick recapitulation of the principal epigenetic regulations of gene expression, it appears evident that these multiple steps are only so many weak points potentially affected by outside influences in the course of organogenesis.

Another interesting emerging hypothesis is the new outlook epigenetics gives people on large-scale chromosomic aberrations. Stepping away from the established concept of establishing parallels between manifestations of a syndrome and the associated deleted/overrepresented genes in linear pathogenic linkage—for example, DiGeorge syndrome and TBX1 deletion included within the 22q11.2 deletion explaining the cardiac involvement—the syndrome could effectively be classified as regulopathy, as for each example, crucial DNA regulation genes are involved and have consequences that have rippling repercussions on more than the affected regions. Down syndrome epigenetic studies have proven that the surnumerous chromosome results in differential expression with upregulation of 27% of genes on chromosome 21, 4.4% of genes on other chromosomes [37]; a concordant study found misregulation of 247 genes not located on chromosome 21 [38]. This is thought to be due to the additional copy of DNMT3L, a methyltransferase present on chromosome 21. In the case of Turner syndrome, haploinsufficiency of KDM6A—a histone demethylase mutated in Kabuki syndrome and known to play a crucial role in cardiac embryogenesis—is thought to be one the mediating elements in the development of heart defects [39].

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