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Commentary

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Cellular regeneration as a potential strategy to treat cardiac conduction disorders

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Diseases of the cardiac conduction system

Formation of the mammalian heart requires differentiation, migration, and complex interactions between cells from different lineages (1). An adult heart consists of more than ten cell types including cardiac and smooth muscle cells, interstitial fibroblasts, endothelial cells, and immune cells (2). When the heart is formed into a well-defined, four-chambered structure during development, the atrioventricular conduction system (AVCS) is derived from neural crest cells between the left and right ventricles (3). These cardiac conduction system (CCS) cells play a critical role in propagating the cardiac impulse from the sinoatrial node, through the atrioventricular (AV) node, and into the His-Purkinje system to ensure coordinated depolarization of the working cardiomyocytes (4).

Disorders of the CCS can be inherited or acquired and lead to cardiac arrhythmias, such as sick sinus syndrome, atrioventricular block (AVB), and bundle branch blocks. Acquired AVB is primarily due to cell injury and death caused by interstitial fibrosis, myocardial ischemia, or interventional procedure complications (4, 5). On the other hand, inherited AVBs can be associated with congenital heart disease, autoimmune disorders, connective tissue disorders, or inherited channelopathies (6, 7). Congenital AVB is reported to be present in 1 of every 20,000 live births, and the prevalence is even higher when considering all cases of AVB during childhood (8, 9). If left untreated, third-degree AVB can lead to asystole and sudden cardiac death (10), which account for approximately 30% of mortalities among patients with congenital AVB (11), highlighting the importance of effective treatment options.

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Conflict of interest: XHTW is a founding partner of Elex Biotech, a start-up company that develops drug molecules to target ryanodine receptors to treat cardiac arrhythmias. XHTW received a consultancy fee from Elex Biotech.

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Current limitations of cardiac pacemaker therapy

Patients with third-degree AVB require continuous ECG monitoring, and external stimulation by implanted devices, such as pacemakers, is advised as a class I therapeutic recommendation (12). The management of fetal and neonatal AVB is more difficult, because pacemaker implantation is impossible in utero or right after birth, so temporary cardiac pacing with an external generator along with pharmacological intervention is the best available option (12). Although generally considered a low-risk procedure, pacemaker implantation is associated with complications such as pneumothorax, infection, damage to blood vessels, and, in the rare, worst-case scenario, death. Furthermore, there are risks associated with the pacemakers, such as lead malfunction, valve adhesions, and lead encapsulation (13). While emerging technologies like ultrasound cardiac stimulation using leadless pacemakers are promising, there is limited information available about their long-term safety and efficacy (14). Moreover, these approaches may not be suitable for the management of congenital AVB.

A major limitation of current device therapies is that they treat the symptom and not the actual causes of CCS cell dysfunction or death (Figure 1). Recent studies show that cardiac plasticity could play pivotal roles in cardiomyocyte regeneration and endogenous repair mechanisms after injury (15). Because cardiac fibroblasts, and even endocardial cells, can differentiate into cardiomyocytes and compensate for cell death following injury (16, 17), understanding the reprogramming mechanisms that underlie cardiac plasticity could provide innovative therapeutic alternatives. Since the CCS has a unique embryologic origin that is distinct from that of the working myocardium (18), understanding

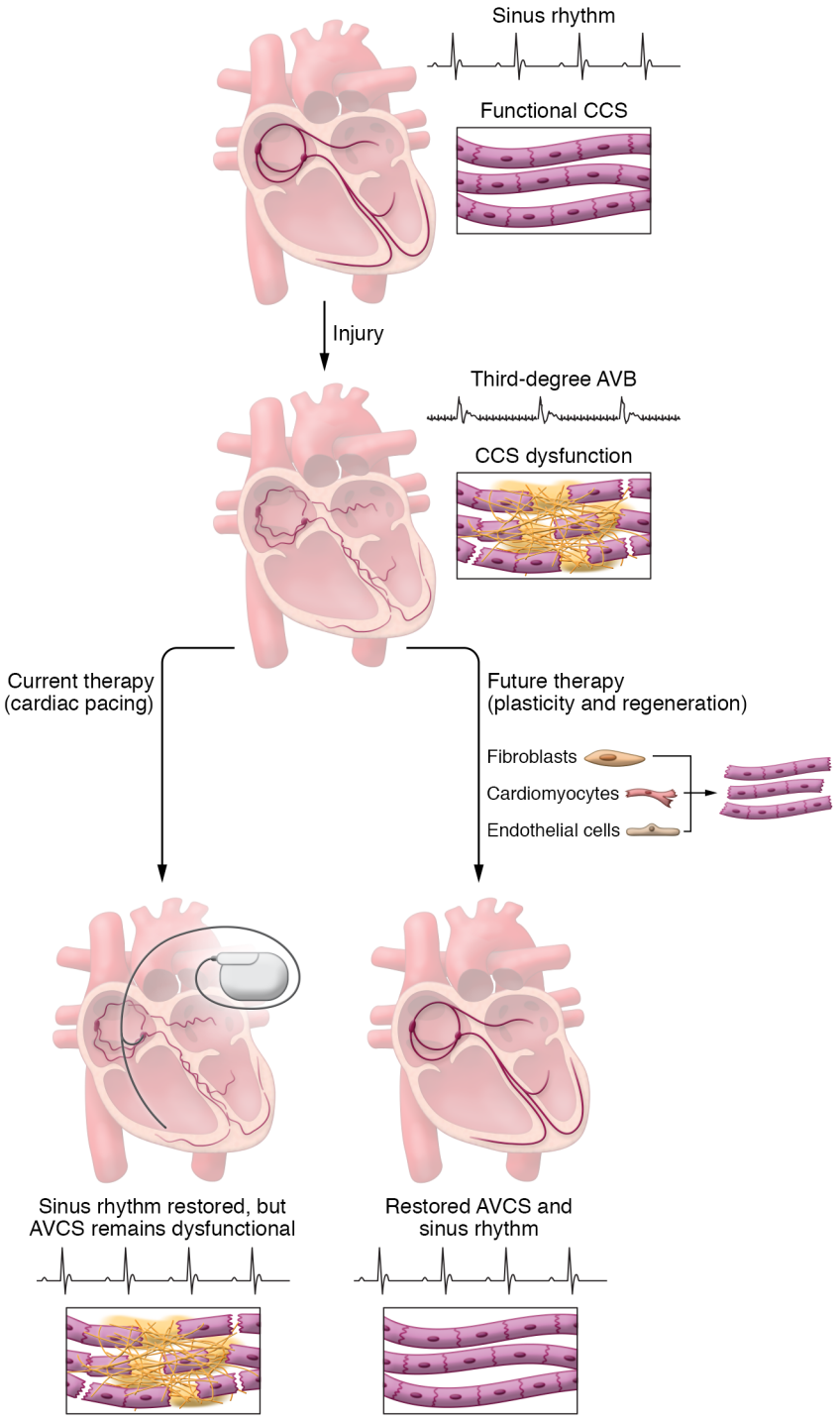

Figure 1. Schematic of cardiac conduction disease and therapeutic options. Schematic of the CCS showing AVCS cells (inset) during normal sinus rhythm or third-degree AVB, which is caused by injury or dysfunction. The current therapy for cardiac conduction disorders often involves cardiac pacing using an electronic pacemaker, but the AVCS cells remain dysfunctional (left). On the other hand, cardiac regeneration and plasticity focus on the restoration of the AVCS cells that are damaged due to injury (right).

how CCS cells respond to injury might uncover the signaling pathways needed to compel other cell types to differentiate into CCS cells.

An inducible cardiac conduction disease model

In this issue of the *JCI*, Wang et al. (19) discovered a paradigm in CCS cell regeneration and plasticity by characterizing a transgenic mouse model of AVB. Under the control of an AVCS-specific enhancer, connexin 30.2 (Cx30.2), which was previously identified by this group (20), the diphtheria toxin fragment A (DTA) transgene was overexpressed specifically within AVCS cells to induce cell damage. Specifically, Wang and authors developed an inducible, transgenic model in which expression of MerCreMer was driven by the Cx30.2 promoter. MerCreMer is a fusion protein of Cre recombinase and mutated ligand-binding domains that requires tamoxifen as a ligand to initiate the translocation of Cre recombinase into the nucleus, where it mediates genomic recombination (21). This mouse allele was crossed with the $Rosa26^{DTA/LacZ}$ allele containing biallelic stop codons that, upon Cre recombinase–mediated excision, led to the expression of both DTA and LacZ, respectively.

When tamoxifen was administered on P49 and P56, the mice developed first-degree AVB (i.e, P-R interval prolongation) up to third-degree AVB (i.e., complete AV dissociation). These mice also developed a progressive deterioration of left ventricular function with a reduction in fractional shortening by 6 months of age. Histological examination revealed the presence of patchy interstitial fibrosis, consistent with the remodeling seen in patients with right ventricular pacing (22). These findings suggest that the authors created a genetic mouse model of inducible AVB that may have advantages over surgical disease models (23) and could be used to study the mechanisms underlying the progression of heart block at different ages.

AVCS regeneration and plasticity

Wang et al. (19) also used their animal model to determine whether young mice could recover from AVCS ablation. When tamoxifen was administered on P0, recovery from first-degree to second-degree AVB to normal sinus rhythm was observed in 40% of the neonatal mice. These findings revealed that AVCS cells possessed a regenerative capacity in neonatal mice that varied depending on the severity of the injury but was lost in older, juvenile mice (P21). To identify which

cell type mediates AVCS regeneration, the authors performed immunostaining of neonatal mouse hearts, which revealed no substantial proliferation of cardiomyocytes, suggesting that noncardiomyocyte cells proliferated following neonatal AVCS injury. Future lineage-tracing studies would be needed to better characterize the origin of the cell types involved in AVCS regeneration. The authors speculate that fusion of noncardiomyocytes with myocytes or migration of transitional atrial-nodal or nodo-ventricular cells into the injury zone might contribute to AVCS repair. One prior study showed that brown adipose tissue–derived mesenchymal stem cells, both in vitro and in vivo, could regenerate into CCS cells (24), suggesting that adipocytes might also contribute to AVCS regeneration.

Future directions

In this study, Wang et al. (19) provide evidence for AVCS regeneration and plasticity, which could have substantial implications for the treatment of cardiac conduction diseases. It will be important to perform follow-up studies aimed at identifying the cell type(s) responsible for AVCS regeneration and any transcriptional programs involved in local cellular plasticity. Next, the authors or other groups could try to reactivate these cell populations and/or signaling pathways in juvenile and adult mice in an effort to extend the currently limited time window of regeneration (Figure 1). At the same time, studies should ensure that no accessory bypass tracts are formed when regeneration is stimulated in older hearts. The data by Wang et al. (19) suggest that neonatal AVCS plasticity does not lead to bypass tract formation, but this abnormal conduction pathway might be more of a concern during exogenously induced regeneration.

Summary and conclusion

Whereas prior studies revealed the regenerative potential of cardiac myocytes (15, 25), it was unknown whether CCS cells, which are a distinct cell type with a different developmental origin, could also proliferate after birth. By inducing AVCS injury in neonatal mice, Wang et al. (19) revealed evidence for AVCS plasticity and regeneration. Their data showed that the functional recovery was not a result of cardiomyocyte proliferation but rather of a distinct regenerative response. Future mechanistic studies may create opportunities to treat cardiac conduction disease using biological repair approaches.

Author contributions

SKL and MMH wrote the manuscript. XHTW revised the manuscript and secured funding. SKL and MMH are co–first authors, and SKL is listed first because he led the manuscript preparation effort.

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