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EDITORIAL COMMENT

Growing With the Flow

Insights Into How Flow Mediates Endocardial Fibroelastosis*

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Endocardial fibroelastosis (EFE) is an uncommon cardiac disease characterized by the thickening of the ventricular endocardium and the presence of profound fibrosis. Biopsy of diseased hearts removed from patients with EFE coupled with histological sections of postmortem heart tissues aptly depict the pathological nature of EFE—dilated or contracted left ventricle (LV) composed of thick layers of opaque and stiff elastic fibers at the endocardium lining, thickened edges of the mitral valve leaflets, and papillary muscle displacement (1,2). Collectively, these gross morphological observations are correlated with poor cardiac functioning resulting in cardiomyopathies, heart failure, and even death. EFE predominantly affects young children and is often observed in tandem with other congenital health conditions such as hypoplastic left heart syndrome (HLHS). HLHS patients with EFE usually have a poor prognosis. The mystery remains—what exactly is causing this aberrant fibrotic growth? A previous study by Xu et al (3) suggested that abnormal endothelial-to-mesenchymal transition (EndoMT) could explain EFE pathogenesis. However, the underlying molecular mechanisms explaining the development of EFE have yet to be fully elucidated. Today, there is no cure for EFE; treatment focuses on ameliorating symptoms and sustaining heart functions. The lack of EFE-specific therapeutic or pharmaceutical interventions is perpetuated by the gap in knowledge pertaining to the pathogenesis of EFE. Therefore, studies investigating the cause of EFE will be valuable endeavors. In this issue of *JACC: Basic to Translational Science,* Oh et al (4) established a neonatal rodent surgical model of EFE with different flow profiles to investigate the pathological effects of abnormal flow condition on EFE development. More importantly, they also identified a potential therapy, losartan, that significantly abrogated EFE in the animal models.

One major highlight of the study is that Oh et al (4) performed neonatal heterotopic heart transplantation to create surgical rat models that recapitulate different blood flow profiles in the left ventricle—static flow (unloaded), regurgitation flow (mimics that of patients with EFE), and normal flow (loaded). These blood flow profiles were confirmed via echocardiogram. At 7 days post-transplantation, the hearts of animals exposed to static and regurgitation flow displayed thickening of LV endocardium. In the absence of normal flow, significant levels of fibrosis and collagen deposition in the LV endocardial and subendocardial layers were also observed. Therefore, the authors were successful in illustrating that rodent hearts exposed to static and regurgitation flow showed morphological similarities to patients with EFE. Next, molecular signatures of hearts exposed to static and regurgitation flows reflect higher levels of EndoMT-specific transcription factors *SNAIL* and *SLUG*, as well as *αSMA*, which marks mesenchymal cells. Protein levels of several endothelial cell (EC) markers such as CD31 and VE-cadherin were downregulated. These findings effectively echo that of Xu et al (3), in that abnormal flow induces EndoMT phenotypes while reducing expression of EC markers in tandem.

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Mechanistically, Oh et al (4) found that transforming growth factor (TGF)-β1 was elevated in static and regurgitation conditions, which was indicative of the role played by the TGF-β/bone morphogenetic protein pathway in flow-mediated EndoMT. To that effect, the authors tested a U.S. Food and Drug Administration–approved angiotensin II receptor blocker, losartan, known to reduce TGF-β expression (5) and for its antifibrotic properties, on the EFE rat models. A total of 7 days of losartan treatment was able to significantly reduce fibrotic area in the LV endocardium of unloaded hearts; several other EndoMT phenotypes were also reversed. This not only further highlights the involvement of the TGF-β/bone morphogenetic protein pathway in regulating flow-mediated EndoMT, but also illustrates the clinical potential of Losartan in reducing fibrotic growth in patients with EFE. However, it is interesting to note that losartan treatment did not restore EC marker expression. Perhaps reducing TGF-β expression alone is insufficient in completely reversing all disease phenotypes or that the dose and/or length of treatment need to be re-evaluated. More studies have to be performed in this regard to determine the effectiveness of losartan in treating EFE. The ultimate efficacy of losartan will be determined in future clinical trials.

To further validate the results in a human system, Oh et al (4), isolated human endocardial endothelial cells (HUEECs) from the LVs of human endocardial tissues. The HUEECs were subjected to laminar shear stress (LSS) to mimic normal blood flow in vivo. Bulk RNA-sequencing was performed to compare transcriptomic changes in HUEECs exposed to LSS vs those exposed to the static condition. Albeit brief, GO term analysis of the sequencing data revealed that genes associated with mesenchymal cell differentiation, heart valve development, and Notch signaling were suppressed in cells exposed to LSS, indicating that lack of normal blood flow (static condition) could trigger EFE development and EndoMT-related gene expression changes. The HUEECs were obtained from endocardial tissues of 3 patients receiving surgical treatment for existing congenital heart diseases and, hence, may contain genetic mutations that alter the physiology of the HUEECs and introduce heterogeneity in RNA-seq analysis in different patient samples. Additionally, although the authors used a “clean” system to assess the impact of flow solely on HUEECs, coculture experiments with cardiomyocytes and fibroblasts may be necessary to evaluate the role(s) played by other cells in flow-mediated EndoMT.

In 2017, compelling evidence provided by a study conducted by Zhang et al (6) pointed towards the contribution of epicardial-to-mesenchymal transition (EpiMT) to fibrosis in EFE. This seemingly contradicts both Xu et al (3), and Oh et al (4), whose data suggest EndoMT as the primary driver of EFE pathogenesis. Zhang et al (6) conducted lineage-tracing experiments that illustrated that EpiMT, not EndoMT, is the main source of fibrosis in EFE. By tracing endocardial cells that express Nfatc1, they showed that only about 6% of fibroblast within the EFE-like tissues in mice were derived from Nfatc1-expressing endocardium. On the other hand, by tracing epicardial cells that express Wt1, about 65% of fibroblasts were derived from Wt1-expressing epicardium. Nonetheless, both Oh et al (4) and Zhang et al (6) were consistent in showing that manipulating TGF-β signaling could reduce fibrosis in EFE rodents. A study recently published by Miao et al (7) showed that endocardial ECs derived from HLHS patient iPSCs also showed down-regulation of EndoMT genes. However, these cells were treated with exogenous TGF-β2 to stimulate EndoMT. Therefore, this leads us to the EndoMT vs EpiMT debate—it remains unclear as to whether EndoMT is the bona fide driver of fibrosis in EFE. Although the data presented by Oh et al (4) support the idea that EndoMT is largely involved in EFE pathogenesis in their particular rat model, they were careful not to exclude other possible molecular mechanisms such as EpiMT.

More experiments will need to be done to resolve the debate. Given the difficulty in lineage tracing in rat models, performing single-cell RNA sequencing of heart samples from the surgical rat models in Oh et al (4) may provide a more in-depth look into the different cell populations altered in unloaded and regurgitation flow conditions. Pseudotime trajectory and RNA velocity analyses may also provide a clue to understand the origin of EFE in rat models. The use of patient with EFE-specific iPSCs may serve as an in vitro platform to study the development of EFE and to screen for compounds that reverse EFE in a personalized manner. In addition, investigating the mechanical sensors involved in sensing the flow in endocardial cells will provide a deeper understanding of how abnormal flow can trigger aberrant fibrosis seen in EFE.

This study holds great translational potential for developing a new therapy for EFE. Oh et al (4) demonstrated that Losartan treatment ameliorated the disease phenotypes exhibited by the surgical rat models, which requires further testing in both large animal models and clinical trials. Also, the study re-emphasizes the need to address the persistent issue of abnormal flow when treating patients with EFE, in addition to other pharmacological interventions.

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Although the association between flow and EndoMT in the context of EFE has already been proposed by others, Oh et al (4) corroborates those findings. This adds to the current controversy on whether EndoMT or EpiMT underlies the rampant fibrogenesis seen in EFE. Certainly, more studies would have to be performed to resolve the debate. Nonetheless, the insights provided by Oh et al (4) allow us to understand the role of abnormal flow condition in causing EFE in rats.

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