



Review

Deciphering the Involvement of the Epicardium in Cardiac Diseases

Rita Carmona ¹, Carmen López-Sánchez ², Virginio García-Martínez ², Virginio García-López ³,
Ramón Muñoz-Chápuli ⁴, Estefanía Lozano-Velasco ⁵ and Diego Franco ^{5,*}

¹ Department of Human Anatomy, Legal Medicine and History of Science, Faculty of Medicine, University of Málaga, 29071 Málaga, Spain; rita@uma.es

² Department of Human Anatomy and Embryology, Faculty of Medicine and Health Sciences, Institute of Molecular Pathology Biomarkers, University of Extremadura, 06006 Badajoz, Spain; clopez@unex.es (C.L.-S.); virginio@unex.es (V.G.-M.)

³ Department of Medical and Surgical Therapeutics, Pharmacology Area, Faculty of Medicine and Health Sciences, University of Extremadura, 06006 Badajoz, Spain; garcialopez@unex.es

⁴ Department of Animal Biology, Faculty of Science, University of Málaga, 29071 Málaga, Spain; chapuli@uma.es

⁵ Cardiovascular Research Group, Department of Experimental Biology, University of Jaén, 23071 Jaén, Spain; evelasco@ujaen.es

* Correspondence: dfranco@ujaen.es

Abstract: The epicardium is a very dynamic cardiac layer with pivotal contributions during cardiogenesis, acting in the postnatal period as an apparently dormant single-cell layer. In mammalian embryos, the epicardium, which originates from the proepicardium, translocates into the pericardial cavity and subsequently rests on the surface of the myocardium. Later, it gives rise to the epicardium-derived cells, which migrate into subepicardial space, invade the developing myocardium, promoting its growth, and contribute to different cell types. Anomalies in the process of epicardial development, the generation of epicardium-derived cells and their signaling mechanisms in different experimental models lead to defective cardiac development, reminiscent of human congenital heart diseases. Furthermore, recent studies have reported that epicardial derivatives in adults, i.e., epicardial adipose tissue, are associated with electrophysiological cardiovascular anomalies. Herein, we provide a state-of-the-art review focusing on both congenital and adult heart diseases associated with epicardial development.

Keywords: epicardium; congenital heart diseases; arrhythmogenic diseases; epicardial adipose tissue



Citation: Carmona, R.; López-Sánchez, C.; García-Martínez, V.; García-López, V.; Muñoz-Chápuli, R.; Lozano-Velasco, E.; Franco, D. Deciphering the Involvement of the Epicardium in Cardiac Diseases. *Hearts* **2023**, *4*, 81–93. <https://doi.org/10.3390/hearts4040011>

Academic Editor: Matthias Thielmann

Received: 15 October 2023
Revised: 7 November 2023
Accepted: 8 November 2023
Published: 10 November 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Origin and Derivatives of the Epicardium

Structurally, the outer layer of the heart is known as the pericardium, consisting of an anatomic cover that also includes the cardiac roots of the great vessels. It is constituted by the fibrous and serosal pericardium layers. The most superficial layer is the fibrous pericardium, formed by a connective tissue that surrounds the heart without being attached to it. The serosal pericardium consists of two layers: the parietal pericardium, directly related to the fibrous pericardium, and the visceral pericardium, also known as the epicardium, the inner layer of the pericardium closely surrounding the underlying myocardium [1].

During development, the epicardium originates from an extracardiac primordium, the proepicardium (PE), which is constituted by a cluster of mesothelial cells located on the pericardial side of the *septum transversum* in mammalian embryos [2–6] (Figure 1). In the early embryo, the epicardium adopts the form of a squamous cell epithelium, which either rests directly on the surface of the myocardium or covers a subepicardial space populated by mesenchymal cells [7]. It has been reported that the PE originates from the periphery of the heart-forming fields in the lateral plate mesoderm (LPM) and is part of an early cardiac progenitor lineage [8]. Interestingly, all the proepicardial cells are morphologically similar,

but with distinct differentiation potential identified via different marker expression [9]. Therefore, the detailed composition and the functional role of the epicardium are not fully known.

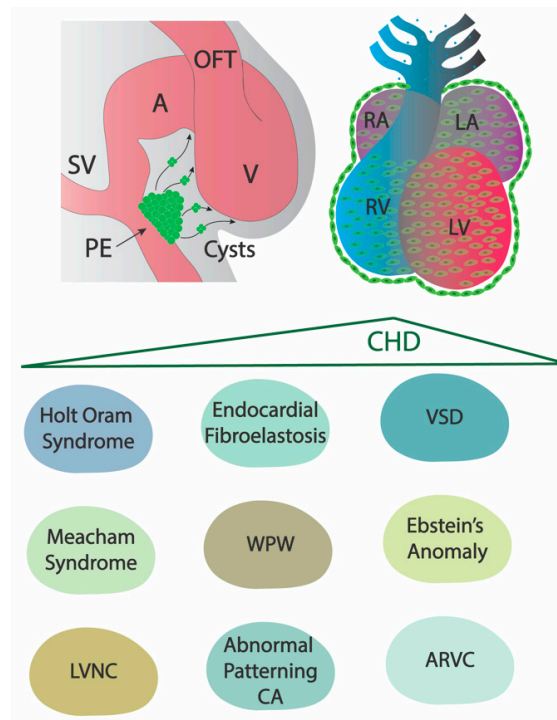


Figure 1. Schematic representation of the origin and development of the embryonic epicardium. The image illustrates the formation of the proepicardium (PE), its migration towards and subsequent lining of the naked embryonic myocardium via cyst delivery into the pericardial cavity. The plausible involvement of defective PE/epicardial development in distinct congenital heart diseases is also depicted. SV, sinus venosus; PE, proepicardium; A, embryonic atrium; V, embryonic ventricle; OFT, outflow tract; RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; CHD, congenital heart disease; CA, coronary arteries; VSD, ventricular septal defect; WPW, Wolf–Parkinson–White syndrome; LVNC, left ventricular non compaction; ARVC, arrhythmogenic right ventricular cardiomyopathy.

After PE formation, PE translocation to the myocardium takes place via the release of free-floating cell clusters (or cysts) into the pericardial cavity in mice [10–13] (Figure 1). After attachment to the myocardial surface, cells start to migrate laterally, until the entire heart is enveloped by the epicardium [4]. Once the epicardium is established, epicardial cells will directly start to participate in the formation of the cardiac walls. A subset of epithelial cells will undergo an epithelial to mesenchymal transition (EMT), giving rise to epicardium-derived cells (EPDCs), which migrate into a matrix-rich subepicardial layer, defining the subepicardium [14–16]. Noticeably, the presence of differences between the atrial and ventricular epicardium during human epicardial development has been reported [17]. Furthermore, relevant differences, both in terms of the overlying epicardial structure and EPDC differentiation potential *ex vivo*, were observed between ventricular and atrial chambers. These data indicate heterogeneous distribution during development and regional contributions to phenotypes underlying human birth defects. From the subepicardium, mesenchymal EPDCs invade the myocardium in a spatiotemporally regulated fashion, in which factors expressed by the underlying myocardium define the likelihood of EPDC migration and also possibly differentiation [16,18]. EPDCs present multi-lineage potential, giving rise to smooth muscle cells (SMCs), which will later form the coronary vessels together with cardiac fibroblasts (CF) in a developed heart [13–18]. Additionally, in mice, EPDCs have been reported to be contributors to cardiomyocytes (CMs) and car-

diac endothelial cells (ECs), although this potential differentiation is still under scientific discussion [13,14,19–22].

Unlike in development, the postnatal mammalian epicardium seems to be a dormant single-cell layer, probably due, at least in part, to the fact that embryonic genes involved in epicardial activation, such as *WT1*, *Tbx18*, *Tcf21* and *Raldh2*, are severely down-regulated [23,24]. However, more recent studies [25] integrating single-cell sequencing and spatial transcriptome technology revealed that several markers, including *Wt1*, *Tbx18*, *Upk1b* and *Upk3b*, were expressed during development in both the epicardium from embryonic epicardial tissue (EET) and the postnatal epicardial tissue (PET). Nevertheless, some other genes, just like *Nnat* and *Tcf21*, presented high expression in EET but not in PET, thus revealing the cellular and temporal heterogeneity of epicardial cells during development. In light of this, a comparative study on human epicardial cells between fetal and adult hearts, at a single-cell resolution [26], showed both compositional and molecular differences between them, revealing, as a fundamental characteristic, that the adult epicardium has a limited population of mesenchymal EPDCs.

Additionally, previous studies have shown, in mice, that the extracellular matrix (ECM) is a crucial factor in EMT and EPDC generation during cardiac development [27]. In this sense, during development, an epicardial gene marker, epidermal growth factor-containing fibulin extracellular matrix protein 1 (*Efemp1*), showed increased and maintained expression in the mature epicardium, thus playing an important role in the mature epicardium by regulating ECM signaling pathways [25].

2. The Role of the Embryonic Epicardium in Adult Structural Heart Diseases

As previously said, the embryonic epicardium plays essential roles in cardiac development, firstly by giving rise to a mesenchymal cell population (EPDC) that migrates into the myocardium and has the capacity to differentiate into cardiac fibroblasts, vascular smooth muscle cells and a small fraction of the coronary endothelium. Secondly, it secretes factors necessary for cardiomyocyte growth and maturation [11]. In this context, EPDCs express a number of FGFs, particularly FGF-9, that induce cardiomyocyte proliferation through FGFR1c and FGFR2c [28]. On the other hand, myocardial FGF-10 signaling through epicardial FGFR2b promotes EPDC invasion and differentiation into fibroblasts [29]. Another epicardial mitogen for cardiomyocytes is IGF-2 [30,31]. These signaling mechanisms are regulated by retinoic acid (RA), which stimulates the epicardial secretion of trophic signals. RA is synthesized in the epicardium by the enzyme RALDH2 [32].

Thus, the developing epicardium is currently recognized as an essential protagonist of cardiac development [33–35]. Anomalies in the process of epicardial development, the generation of EPDCs and their signaling mechanisms could be responsible for defective cardiac development contributing to congenital heart disease (CHD). It is estimated that almost 8 million children are born annually in the world with congenital defects (CD), and CHD comprises the most frequently occurring severe congenital malformations, affecting 8.2 per 1000 newborns in the world [36].

At present, there is no clear evidence of the involvement of a defective epicardial development in human CHD. However, a number of animal models displaying anomalies in the formation of the epicardium or EPDCs show phenotypes that can be linked to the pathophysiology of some CHDs (Figure 1). We will review some reports that suggest a potential association between structural defects of the adult heart and epicardial maldevelopment.

2.1. Holt–Oram Syndrome

Holt–Oram syndrome is an autosomal dominant condition considered a rare disease. This syndrome is caused by mutations in the *TBX5* gene and it is characterized by structural cardiac abnormalities, the commonest including atrial and/or ventricular septal defects [37,38]. *Tbx5* is dynamically expressed in epicardial progenitors and their lineages during embryonic development. The conditional deletion of *Tbx5* in the epicardium of mouse embryos provoked a significantly reduced ratio of newborn mutants [39]. *Tbx5* deficiency did not alter proepicardial cell proliferation, apoptosis or polarity, but the epi-

cardium showed deficient attachment to the cardiac surface and reduced proliferation. Despite the normal expression of EMT markers, mutant embryos showed a strong reduction in the number of EPDCs invading the myocardium, a decrease in vascular smooth muscle cell recruitment and a smaller number of cardiac fibroblasts. Since these cells are essential for coronary development, the cardiac vasculature was also impaired, with a 45% reduction in PECAM-1-positive capillary density in mutant hearts. Cardiomyocyte proliferation was also reduced by about a half in E12.5 mutant embryos compared to that in wildtype embryos, and myocardial apoptosis was observed in E14.5 mutant embryos. Muscular and membranous ventricular septal defects were seen in 15% of E15.5 mutant embryos. Interestingly, these features, a thin myocardium and ventricular septal defect, are reminiscent of Holt–Oram syndrome. Thus, the impaired function of TBX5 in the epicardium could be responsible for a significant part of the phenotype seen in human Holt–Oram syndrome patients. This phenotype could be caused by anomalies in the main roles normally played by EPDCs, their contribution to coronary development and the production of paracrine growth factors for the myocardium.

2.2. Meacham Syndrome

Meacham syndrome is an extremely rare condition characterized by abnormal genitourinary development, complex congenital heart disease and diaphragmatic abnormalities. The only genetic anomaly hitherto associated with Meacham syndrome has been mutations in Wilms' tumor suppressor gene (*WT1*) [40]. Two patients, who died six days and three years after birth, had heterozygous missense mutations in *WT1*, namely Arg366Cys (exon 8) and Arg394Trp (exon 9). Since *Wt1* is expressed in the embryonic epicardium, the authors of this study suggested that the cardiac defects observed in patients with Meacham syndrome, mainly ventricular and atrial septal defects, could be related to the defective development of the epicardium and EPDCs. However, further information will be necessary to test this hypothesis, since the two patients with Meacham syndrome bearing *WT1* mutations showed no congenital cardiac defects. Intriguingly, the very same mutations were found in two patients with Denys–Drash syndrome, a disease caused by *WT1* gene mutations characterized by the impaired development of the kidneys and reproductive organs, diffuse glomerulosclerosis and gonadal dysgenesis. The identity of the mutations in Meacham and DDS points to some phenotypic overlap between these syndromes. Since congenital heart defects are normally absent in Denys–Drash syndrome, the relationship between *WT1* mutations in the epicardium and Meacham syndrome remains a hypothesis.

2.3. Left Ventricular Non-Compaction

As we have described above, several FGFs and IGF-2 secreted by the epicardium are potent mitogens that regulate cardiomyocyte proliferation in the embryonic ventricular wall. For that reason, a number of animal models of defective epicardial development show a thinning of the ventricular wall, a phenotype that might be related to the human condition known as left ventricular non-compaction.

For example, β -catenin deletion in the proepicardium results in a thin ventricular myocardium and embryonic death [41]. The mucin-like transmembrane glycoprotein podoplanin is expressed by the epicardium, and its loss of function causes defective epicardial development together with a hypoplastic, perforated, compact and septal myocardium [42]. The epicardial deletion of Wilms' tumor suppressor gene (*Wt1*) also results in a thin ventricular myocardium. The epicardial deletion of *Notch1* disrupts coronary artery differentiation and also reduces ventricular wall thickness and myocyte proliferation. More recently, the expression of the angiogenic hormone, prokineticin-2 and its G-protein-coupled receptor PKR1 in the epicardium has been reported as required for normal cardiac development. Their dysfunction can have consequences on the structure and function of the adult heart. The conditional deletion of PKR1 in the murine epicardium causes partial embryonic and postnatal lethality. Adult survivors display a thinner ventricular wall, ischemic cardiomyopathy and systolic dysfunction [43]. Finally, collagen- and calcium-

binding EGF-like domain 1 (CCBE1), a secreted extracellular matrix protein, is expressed by embryonic epicardial cells. CCBE1 knockout mice show thin and hyper-trabeculated ventricular myocardium [44]. In mutant hearts, epicardial and myocardial proliferation decreases, the number of EPDCs invading the myocardium is reduced and EMT markers are deregulated.

Not all the genetic alterations in the epicardial development result in a thinner ventricular wall. NFATC1, a transcription factor of the NFAT family, is expressed in the embryonic epicardium and it has a crucial role in promoting the invasion by EPDCs of the myocardium. The epicardial deletion of NFATC1 impairs the migration of EPDCs and leads to an abnormally compact ventricular wall defect in vessels and fibrous tissue [45]. These embryos show a decreased expression of cathepsin K, an enzyme necessary for extracellular matrix degradation and cell invasion. In this case, the epicardium is able to promote myocardial growth, but EPDCs fail in the invasion of the cardiac wall.

2.4. Endocardial Fibroelastosis

Endocardial fibroelastosis (EFE) is a disease that affects infants and children, although rarely appears in adults. This disease consists of severe ventricular endocardium thickening due to the abundance of subendocardial fibrous tissue composed of collagen and elastin. It was thought that this fibroelastic tissue derived from endocardial cells underwent an aberrant endothelial-to-mesenchymal transition. A recent study in a murine model of EFE demonstrated that neonatal endocardial cells do not contribute to the fibroblasts causing the disease. Lineage tracing of embryonic epicardial-derived cells suggested that they served as the major source of EFE fibroblasts [46]. These authors observed an upregulation of TGF β signaling in the epicardial-derived cells of the EFE murine model. The TGF β antagonist BMP7 reduced fibroblast accumulation and fibrosis in the mice. Thus, the deregulation of embryonic epicardium-derived cells can be responsible for this disease in humans.

2.5. Abnormal Patterning of the Coronary Arteries

The abnormal patterning of coronary vascularization can also be related with anomalies in epicardial development. CCBE1 is an extracellular matrix protein involved in the migration of lymphatic endothelial cells, and also in angiogenesis. CCBE1 is expressed in the embryonic epicardium and sinus venosus, and its loss of function causes the defective development of coronary vessels as well as the delayed development of the coronary artery trunks and the anomalous position of the coronary orifices in the aorta [44]. *Ccbe1* is first expressed in the epicardium and it accumulates over the ventricular surface, participating in the maturation of VEGF-C propeptides. In this way, the embryonic epicardium could be involved in anomalies similar to those in the condition known as low origin of the coronary arteries (LOCA) [47].

2.6. Arrhythmogenic Right Ventricular Cardiomyopathy

The epicardium has been considered relevant to the pathophysiology of arrhythmogenic right ventricular cardiomyopathy, a congenital disorder that causes progressive replacement of the right ventricular myocardium by fibrofatty tissue. This disease is caused by mutations in a number of genes, most of them coding for desmosomal proteins. The desmosomal protein plakophilin-2 (PKP2) is expressed in the embryonic epicardium and EPDCs. PKP2 knockdown in epicardial explants from neonatal rat hearts increases proliferation, differentiation into smooth muscle cells and also the expression and accumulation of lipid markers. These observations suggest a relationship between anomalies in PKP2 expression in the early epicardium and the pathophysiology of arrhythmogenic right ventricular cardiomyopathy [48].

Desmoplakin is another desmosomal protein expressed in the myocardium and epicardium. A conditional heterozygous mutation in the epicardium of desmoplakin in newborns provoked premature death, cardiac dysfunction, arrhythmias, myocardial fibro adipogenesis and apoptosis [49]. A haploinsufficiency of desmoplakin in the epicardial

cells caused cardiac dilatation and dysfunction at 6 months of age, with a reduction in left ventricular wall thickness, fractional shortening and ejection fraction. In addition, mutant mice exhibited paroxysmal supraventricular tachycardia, a ventricular trigeminal rhythm and second-degree atrioventricular blocks. Epicardial cells with a heterozygous deletion of desmoplakin gave rise to clusters of EPDCs expressing paracrine factors, including TGF β 1 and FGFs, which have well-established roles in the pathogenesis of myocardial pathology, including arrhythmogenic right ventricular cardiomyopathy [50].

Thus, anomalies in the expression of desmosomal proteins by embryonic or very early epicardial cells cause myocardial fibro-adipogenesis and cardiac dysfunction, contributing eventually to the pathogenesis of arrhythmogenic cardiomyopathy.

2.7. Ventricular Septal Defect

The developing epicardium has also been related to the normal process of ventricular septation [51,52]. During development, the anterior wall of the ventricle folds, forming an interventricular septum and trapping the epicardium in the midline. The infolded myocardium contributes to the anterior part of the interventricular septum. The function of these epicardial cells is uncertain, but some models of defective epicardial development in mice and chickens display defective interventricular septum formation. Thus, defective epicardial development might also be connected with anomalies in the interventricular septum.

2.8. Ebstein's Anomaly and Wolff–Parkinson–White (WPW) Syndrome

Ebstein's anomaly is a rare congenital defect that affects the tricuspid valve. Valve leaflets are abnormally shaped or the valve is placed lower than normal. Part of the migrating EPDCs populate the subvalvular ventricular myocardium at the level where it delaminates and forms tendinous chords. Impaired EPDC migration might cause anomalies in this process, leading to tricuspid valve displacement, as seen in Ebstein's anomaly [4]. On the other hand, EPDC-derived fibroblasts contribute to the annulus fibrosus tissue that insulates the atrioventricular junction. Impaired migration of EPDCs causes the persistence of myocardial accessory atrioventricular pathways and ventricular preexcitation [53]. This is a characteristic of Wolff–Parkinson–White syndrome, which is often associated with Ebstein's anomaly and sometimes to ventricular non-compaction. It is interesting that severe AV endocardial cushion defects and a thin myocardium also appear after the epicardial deletion of transcriptional regulator FOG-2 in mice. All these data suggest that abnormal epicardial development can be related to structural defects in the atrioventricular canal.

3. The Role of the Embryonic Epicardium in Adult Electrophysiological Heart Diseases

As previously stated, the embryonic epicardium give rise to distinct cardiovascular cell types during cardiogenesis. Verhuele et al. [54] nicely reported that the loss of epithelial continuity in the epicardial layer, as a consequence of endomyocardial fibrosis, led to increased complexity in atrial fibrillatory conduction, supporting a causative link between the epicardium and atrial fibrillation (AF). More recently, Yamaguchi et al. [55] nicely demonstrated, using a Tbx18Cre driver in mice, that the adipose tissue around the heart is also an epicardial derivative, and for that reason is regarded as epicardial adipose tissue (EAT). Furthermore, these authors demonstrated that, although the extent of cardiac adipose tissue is consistently different in mice and humans, epicardial cells from both species have the capacity of transforming into adipocytes in vitro, a process that is mediated by PPAR γ .

Over the last decade, we have witnessed compelling evidence of the causal relationship between EAT deposition and the occurrence of distinct cardiac electrophysiological pathologies, particularly atrial fibrillation [56–62] (Figure 2). It is important to highlight in this context that EAT deposition has been related to AF incidence in association with both ST elevation (STEMI) [63] and non-ST elevation (NSTEMI) in myocardial infarction patients [64]. Atrial EAT volume, measured via multidetector computed tomography, has

also been directly related to distinct types of AF in the context of coronary artery diseases (CAD) [65]. Curiously, these authors also reported that ventricular EAT volume was also significantly associated with CAD, irrespective of AF, supporting the notion that an uneven chamber-specific distribution of EAT might be direct related to the pathogenesis of AF or CAD, respectively, probably influencing local inflammatory processes [65]. EAT volume and/or thickness, measured via computed tomography or transthoracic ecocardiography, are also associated with isolated permanent AF [66], as well as with AF recurrence after ablation [67,68], coronary artery bypass [69], hemodialysis [70] or an AF adverse outcome, particularly chronic heart failure [71]. Besides AF, EAT deposition has also been related to atrial conduction abnormalities [72,73], characterized by slower conduction, greater electrocardiogram fractionation, increased fibrosis and impaired subcellular localization of connexin 40, which becomes prominently distributed in the lateral cardiomyocyte sides. Additionally, atrial dysfunction in the absence of CAD and/or AF is also associated with EAT deposition [60].

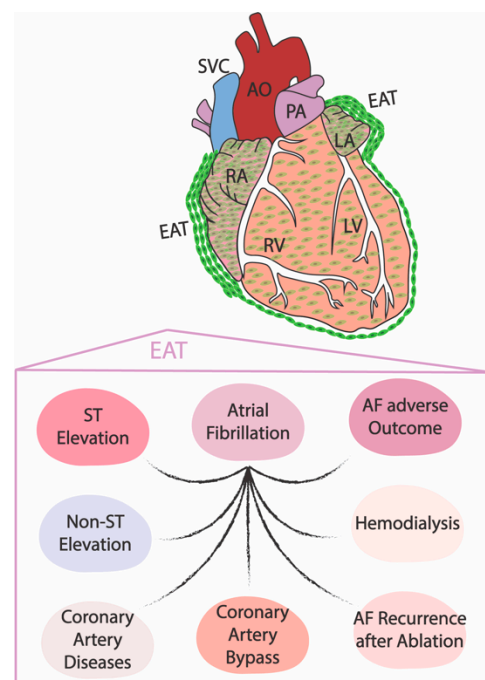


Figure 2. Schematic representation of the contribution of the epicardial adipose tissue to cardiac diseases. The image illustrates the lining of the adult epicardium, its contribution to the epicardial adipose tissue (EAT), which is particularly abundant in the atrial region. Similarly, the correlation of abnormal EAT deposition and/or EAT signaling in relation to atrial fibrillation in distinct clinical settings is also shown. RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; SVC, superior vena cava; AO, aorta; PA, pulmonary artery; EAT, epicardial adipose tissue.

An understanding of the cellular and molecular mechanisms that link excessive EAT deposition and distinct cardiac electrophysiological pathologies is progressively emerging. Several authors have reported that distinct signaling pathways emanating from EAT can serve as a substrate to increase atrial fibrosis and/or fibrofatty infiltration, thus enhancing the vulnerability to AF [74,75]. In particular, Abe et al. [74] highlighted the relevance of increased macrophage infiltration, the upregulation of proinflammatory markers such as interleukin-6 (IL6) and tumor necrosis α (TNF α), angiogenic growth factors such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases in EAT, demonstrating that fibrotic remodeling and cytokine/chemokines release from EAT are associated with atrial myocardium fibrosis, a well-known substrate of AF. This same group of researchers further demonstrated that Angptl2, a previously reported cytokine highly expressed in EAT, played a crucial role promoting EAT-induced atrial inflammation and fibrosis [75]. Wang

et al. [76] analyzed the adipokine expression level of EAT in sinus rhythm and AF patients, demonstrating that the expression of connective tissue growth factor (cTGF) is significantly higher in AF patients. Importantly, such an increased expression of cTGF is exclusively observed in EAT samples, as compared to other fat depots such as the subcutaneous adipose tissue (SAT) or the paracardial adipose tissue (PAT) of the same AF patients.

Similarly, inflammatory processes in EAT have been recently associated with AF onset [77–80]. EAT adiponectin signaling [81] and EAT-driven endothelial dysfunction [82] can influence AF vulnerability. In this context, Liu et al. [80] demonstrated that interleukin-1 β expression was significantly increased, while adiponectin was decreased in EAT samples, but not in the serum, of persistent AF patients. Additional evidence of the role of EAT-derived inflammatory signaling was reported by Li et al. [81] in an experimental model of rapid atrial pacing (RAP) in dogs. These authors demonstrated that canine RAP leads to increased reactive oxygen species (ROS) accumulation, the upregulation of nuclear factor kappa-B (NF- κ B), IL6 and TNF α , and in transforming growth factor β 1 (TGF β 1) expression in both the left atrial tissues as well as in EAT. PPAR γ and adiponectin on the other hand were significantly downregulated in EAT. Importantly, the expression of these molecules was reversed via metformin administration, correlating with an improvement in electrophysiological parameters. In vitro experiments further demonstrated that adiponectin administration reduced the inflammatory response as well as sarcoplasmic reticulum calcium-handling dysfunction. Importantly, these molecular mechanisms can be mediated by extracellular vesicles emanating from EAT, thus facilitating atrial fibrillation [83], a process that might be mediated by complex lncRNA-microRNA gene regulatory networks as recently reported by Liu et al. [84] and further supported by differential ncRNA expression in EAT from AF patients [85].

Importantly, evidence of distinct EAT interventions, such as botulinum toxin injection or statin treatment, can influence the recurrence of atrial fibrillation [86,87], opening up new therapeutic avenues.

4. Conclusions and Perspectives

Over the last decades, we have witnessed increasing evidence of the functional role of the embryonic epicardium, not only as a source of multiple cardiovascular cell types, such as cardiac fibroblasts and distinct vascular cells, but also as a key signaling layer for the proper development of the myocardial chambers (for a recent review, see [88]). Furthermore, increasing evidence demonstrated that an impairment of the epicardium or epicardial-derived cells leads to cardiac abnormalities. Therefore, the plausible implication of the embryonic epicardium in distinct congenital heart diseases, as reported in this review, might not be surprising.

Our current understanding of the underlying molecular mechanisms remains poorly explored. However, the advent of novel technological tools such as single-cell and spatial transcriptomics will certainly enhance our understanding [24,25,89–91]. In this context, single-cell transcriptomics have already revealed substantial gene expression differences between human embryonic and postnatal epicardial cells [24,25] and identified novel players in epicardial EMT [89,91]. Additionally, the generation of novel experimental models based on organoids or spheroids, but specifically applied to the multipotential epicardium, i.e., epicardioids, as reported by Meier et al. [92], will further widen our understanding of the contribution of the epicardium to cardiac diseases.

In recent years, the contribution of the epicardium has been extended to the epicardial adipose tissues adjacent to the adult heart. Importantly, abundant evidence has been reported on the correlation between epicardial adipose tissue deposition and the occurrence of electrophysiological abnormalities, particularly atrial fibrillation. Noticeably, emerging molecular mechanisms have been already reported in this context, suggesting a pivotal role for inflammation and fibrosis as key triggering mechanisms of electrophysiological disorders of the adjacent atrial myocardium. To date, no single-cell or spatial transcriptomic analyses of EAT have been performed in AF patients. However, a seminal work by Zhou

et al. [93] reported six distinct EAT cell populations in heart failure patients, and identified the role of impaired zinc and copper ion homeostasis. In the next coming years, we will witness increasing evidence of the pivotal role of the embryonic epicardium and its developmental and adult derivatives, in distinct cardiovascular diseases, therefore challenging the classical notion that they constitute a dormant single-cell layer.

Author Contributions: Conceptualization, D.F.; data curation, R.C., C.L.-S., V.G.-M., V.G.-L., R.M.-C., E.L.-V. and D.F.; writing—original draft preparation, R.C., C.L.-S., V.G.-M., V.G.-L., R.M.-C., E.L.-V. and D.F.; writing—review and editing, R.C., C.L.-S., V.G.-M., V.G.-L., R.M.-C., E.L.-V. and D.F.; funding acquisition, D.F. and R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by grants from Junta de Andalucía to DF (CTS446) and from Agencia Estatal de Investigación, Ministry of Science and Innovation of the Spanish Government, to DF and RC (PID2022-138163OB-C32).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hoit, B.D. Anatomy and Physiology of the Pericardium. *Cardiol. Clin.* **2017**, *35*, 481–490. [[CrossRef](#)] [[PubMed](#)]
2. Komiyama, M.; Ito, K.; Shimada, Y. Origin and development of the epicardium in the mouse embryo. *Anat. Embryol.* **1987**, *176*, 183–189. [[CrossRef](#)] [[PubMed](#)]
3. Männer, J.; Perez-Pomares, J.M.; Macias, D.; and Munoz-Chapuli, R. The origin, formation and developmental significance of the epicardium: A review. *Cells Tissues Organs* **2001**, *169*, 89–103. [[CrossRef](#)] [[PubMed](#)]
4. Lie-Venema, H.; van den Akker, N.M.; Bax, N.A.; Winter, E.M.; Maas, S.; Kekarainen, T.; Hoeben, R.C.; deRuiter, M.C.; Poelmann, R.E.; Gittenberger-de Groot, A.C. Origin, fate, and function of epicardium-derived cells (EPDCs) in normal and abnormal cardiac development. *Sci. World J.* **2007**, *7*, 1777–1798. [[CrossRef](#)] [[PubMed](#)]
5. Carmona, R.; Guadix, J.A.; Cano, E.; Ruiz-Villalba, A.; Portillo-Sánchez, V.; Pérez-Pomares, J.M.; Muñoz-Chápuli, R. The embryonic epicardium: An essential element of cardiac development. *J. Cell Mol. Med.* **2010**, *14*, 2066–2072. [[CrossRef](#)] [[PubMed](#)]
6. Niderla-Bielińska, J.; Jankowska-Steifer, E.; Flaht-Zabost, A.; Gula, G.; Czarnowska, E.; Ratajska, A. Proepicardium: Current Understanding of its Structure, Induction, and Fate. *Anat. Rec.* **2019**, *302*, 893–903. [[CrossRef](#)]
7. Muñoz-Chápuli, R.; Macías, D.; González-Iriarte, M.; Carmona, R.; Atencia, G.; Pérez-Pomares, J.M. The epicardium and epicardial-derived cells: Multiple functions in cardiac development. *Rev. Esp. Cardiol.* **2002**, *55*, 1070–1082. [[CrossRef](#)]
8. Mommersteeg, M.T.; Domínguez, J.N.; Wiese, C.; Norden, J.; de Gier-de Vries, C.; Burch, J.B.; Kispert, A.; Brown, N.A.; Moorman, A.F.; Christoffels, V.M. The sinus venosus progenitors separate and diversify from the first and second heart fields early in development. *Cardiovasc. Res.* **2010**, *87*, 92–101. [[CrossRef](#)]
9. Katz, T.C.; Singh, M.K.; Degenhardt, K.; Rivera-Feliciano, J.; Johnson, R.L.; Epstein, J.A.; Tabin, C.J. Distinct compartments of the proepicardial organ give rise to coronary vascular endothelial cells. *Dev. Cell.* **2012**, *22*, 639–650. [[CrossRef](#)]
10. Plavicki, J.S.; Hofsteen, P.; Yue, M.S.; Lanham, K.A.; Peterson, R.E.; Heideman, W. Multiple modes of proepicardial cell migration require heartbeat. *BMC Dev. Biol.* **2014**, *14*, 18. [[CrossRef](#)]
11. Cao, Y.; Duca, S.; Cao, J. Epicardium in Heart Development. *Cold Spring Harb. Perspect. Biol.* **2020**, *12*, a037192. [[CrossRef](#)] [[PubMed](#)]
12. Li, J.; Miao, L.; Zhao, C.; Shaikh Qureshi, W.M.; Shieh, D.; Guo, H.; Lu, Y.; Hu, S.; Huang, A.; Zhang, L.; et al. CDC42 is required for epicardial and pro-epicardial development by mediating FGF receptor trafficking to the plasma membrane. *Development* **2017**, *144*, 1635–1647. [[CrossRef](#)] [[PubMed](#)]
13. Sanchez-Fernandez, C.; Rodriguez-Outeiriño, L.; Matias-Valiente, L.; Ramirez de Acuña, F.; Hernandez-Torres, F.; Lozano-Velasco, E.; Dominguez, J.N.; Franco, D.; Aranega, A.E. Regulation of Epicardial Cell Fate during Cardiac Development and Disease: An Overview. *Int. J. Mol. Sci.* **2022**, *23*, 3220. [[CrossRef](#)] [[PubMed](#)]
14. Smits, A.M.; Dronkers, E.; Goumans, M.J. The epicardium as a source of multipotent adult cardiac progenitor cells: Their origin, role and fate. *Pharmacol. Res.* **2018**, *127*, 129–140. [[CrossRef](#)] [[PubMed](#)]
15. Vrancken Peeters, M.-P.F.M.; Gittenberger-de Groot, A.C.; Mentink, M.M.T.; and Poelmann, R.E. Smooth muscle cells and fibroblasts of the coronary arteries derive from epithelial-mesenchymal transformation of the epicardium. *Anat. Embryol.* **1999**, *199*, 367–378. [[CrossRef](#)] [[PubMed](#)]
16. Lie-Venema, H.; Eralp, I.; Maas, S.; Gittenberger-de Groot, A.C.; Poelmann, R.E.; and DeRuiter, M.C. Myocardial heterogeneity in permissiveness for epicardium-derived cells and endothelial precursor cells along the developing heart tube at the onset of coronary vascularization. *Anat. Rec.* **2005**, *282A*, 120–129. [[CrossRef](#)] [[PubMed](#)]
17. Risebro, C.A.; Vieira, J.M.; Klotz, L.; Riley, P.R. Characterisation of the human embryonic and foetal epicardium during heart development. *Development* **2015**, *142*, 3630–3636. [[CrossRef](#)] [[PubMed](#)]

18. Gittenberger-de Groot, A.C.; Vrancken Peeters, M.-P.F.M.; Mentink, M.M.T.; Gourdie, R.G.; and Poelmann, R.E. Epicardium-derived cells contribute a novel population to the myocardial wall and the atrioventricular cushions. *Circ. Res.* **1998**, *82*, 1043–1052. [[CrossRef](#)]
19. Cai, C.L.; Martin, J.C.; Sun, Y.; Cui, L.; Wang, L.; Ouyang, K.; Yang, L.; Bu, L.; Liang, X.; Zhang, X.; et al. A myocardial lineage derives from Tbx18 epicardial cells. *Nature* **2008**, *454*, 104–108. [[CrossRef](#)]
20. Christoffels, V.M.; Grieskamp, T.; Norden, J.; Mommersteeg, M.T.; Rudat, C.; Kispert, A. Tbx18 and the fate of epicardial progenitors. *Nature* **2009**, *458*, E8–E9, discussion E9–E10. [[CrossRef](#)]
21. Rudat, C.; Kispert, A. Wt1 and epicardial fate mapping. *Circ. Res.* **2012**, *111*, 165–169. [[CrossRef](#)] [[PubMed](#)]
22. Carmona, R.; Barrena, S.; López Gambero, A.J.; Rojas, A.; Muñoz-Chápuli, R. Epicardial cell lineages and the origin of the coronary endothelium. *FASEB J.* **2020**, *43*, 5223–5239. [[CrossRef](#)] [[PubMed](#)]
23. Smart, N.; Bollini, S.; Dubé, K.N.; Vieira, J.M.; Zhou, B.; Davidson, S.; Yellon, D.; Riegler, J.; Price, A.N.; Lythgoe, M.F.; et al. De novo cardiomyocytes from within the activated adult heart after injury. *Nature* **2011**, *474*, 640–644. [[CrossRef](#)] [[PubMed](#)]
24. Braitsch, C.M.; Combs, M.D.; Quaggin, S.E.; Yutzey, K.E. Pod1/Tcf21 is regulated by retinoic acid signaling and inhibits differentiation of epicardium-derived cells into smooth muscle in the developing heart. *Dev. Biol.* **2012**, *368*, 345–357. [[CrossRef](#)] [[PubMed](#)]
25. Du, J.; Yuan, X.; Deng, H.; Huang, R.; Liu, B.; Xiong, T.; Long, X.; Zhang, L.; Li, Y.; She, Q. Single-cell and spatial heterogeneity landscapes of mature epicardial cells. *J. Pharm. Anal.* **2023**, *13*, 894–907. [[CrossRef](#)]
26. Knight-Schrijver, V.R.; Davaapil, H.; Bayraktar, S.; Ross, A.D.B.; Kanemaru, K.; Cranley, J.; Dabrowska, M.; Patel, M.; Polanski, K.; He, X.; et al. A single-cell comparison of adult and fetal human epicardium defines the age-associated changes in epicardial activity. *Nat. Cardiovasc. Res.* **2022**, *1*, 1215–1229. [[CrossRef](#)]
27. Lupu, I.E.; Redpath, A.N.; Smart, N. Spatiotemporal Analysis Reveals Overlap of Key Proepicardial Markers in the Developing Murine Heart. *Stem Cell Rep.* **2020**, *14*, 770–787. [[CrossRef](#)]
28. Lavine, K.J.; Yu, K.; White, A.C.; Zhang, X.; Smith, C.; Partanen, J.; Ornitz, D.M. Endocardial and epicardial derived FGF signals regulate myocardial proliferation and differentiation in vivo. *Dev. Cell.* **2005**, *8*, 85–95. [[CrossRef](#)]
29. Vega-Hernández, M.; Kovacs, A.; De Langhe, S.; Ornitz, D.M. FGF10/FGFR2b signaling is essential for cardiac fibroblast development and growth of the myocardium. *Development* **2011**, *138*, 3331–3340. [[CrossRef](#)]
30. Li, P.; Cavallero, S.; Gu, Y.; Chen, T.H.; Hughes, J.; Hassan, A.B.; Brüning, J.C.; Pashmforoush, M.; Sucov, H.M. IGF signaling directs ventricular cardiomyocyte proliferation during embryonic heart development. *Development* **2011**, *138*, 1795–1805. [[CrossRef](#)]
31. Shen, H.; Cavallero, S.; Estrada, K.D.; Sandovici, I.; Kumar, S.R.; Makita, T.; Lien, C.L.; Constancia, M.; Sucov, H.M. Extracardiac control of embryonic cardiomyocyte proliferation and ventricular wall expansion. *Cardiovasc. Res.* **2015**, *105*, 271–278. [[CrossRef](#)] [[PubMed](#)]
32. Guadix, J.A.; Ruiz-Villalba, A.; Lettice, L.; Velecela, V.; Muñoz-Chápuli, R.; Hastie, N.D.; Pérez-Pomares, J.M.; Martínez-Estrada, O.M. Wt1 controls retinoic acid signalling in embryonic epicardium through transcriptional activation of Raldh2. *Development* **2011**, *138*, 1093–1097. [[CrossRef](#)] [[PubMed](#)]
33. Ridge, L.A.; Mitchell, K.; Al-Anbaki, A.; Qureshi, W.M.S.; Stephen, L.A.; Tenin, G.; Lu, Y.; Lupu, I.E.; Clowes, C.; Robertson, A.; et al. Non-muscle myosin IIB (Myh10) is required for epicardial function and coronary vessel formation during mammalian development. *PLOS Genet.* **2017**, *30*, e1007068. [[CrossRef](#)] [[PubMed](#)]
34. Marques, I.J.; Ernst, A.; Arora, P.; Vianin, A.; Hetke, T.; Sanz-Morejón, A.; Naumann, U.; Odriozola, A.; Langa, X.; Andrés-Delgado, L.; et al. Wt1 transcription factor impairs cardiomyocyte specification and drives a phenotypic switch from myocardium to epicardium. *Development.* **2022**, *149*, dev200375. [[CrossRef](#)] [[PubMed](#)]
35. Boezio, G.L.M.; Zhao, S.; Gollin, J.; Priya, R.; Mansingh, S.; Guenther, S.; Fukuda, N.; Gunawan, F.; Stainer, D.Y.R. The developing epicardium regulates cardiac chamber morphogenesis by promoting cardiomyocyte growth. *Dis. Model. Mech.* **2023**, *16*, dmm049571. [[CrossRef](#)] [[PubMed](#)]
36. Liu, Y.; Chen, S.; Zuhlke, L.; Black, G.C.; Choy, M.K.; Li, N.; Keavney, B.D. Global birth prevalence of congenital heart defects 1970-2017: Updated systematic review and meta-analysis of 260 studies. *Int. J. Epidemiol.* **2019**, *48*, 455–463. [[CrossRef](#)] [[PubMed](#)]
37. Basson, C.T.; Cowley, G.D.; Solomon, S.D.; Weissman, B.; Poznanski, A.K.; Traill, T.A.; Seidman, J.G.; Seidman, C.E. The clinical and genetic spectrum of the Holt-Oram syndrome. *N. Engl. J. Med.* **1994**, *330*, 885–891. [[CrossRef](#)] [[PubMed](#)]
38. Sletten, L.J.; Pierpont, M.E. Variation in severity of cardiac disease in Holt-Oram syndrome. *Am. J. Med. Genet.* **1996**, *65*, 128–132. [[CrossRef](#)]
39. Diman, N.Y.S.G.; Brooks, G.; Kruithof, B.P.T.; Elemento, O.; Seidman, J.G.; Seidman, C.E.; Basson, C.T.; Hatcher, C.J. Tbx5 is required for avian and Mammalian epicardial formation and coronary vasculogenesis. *Circ. Res.* **2014**, *115*, 834–844. [[CrossRef](#)]
40. Suri, M.; Kelehan, P.; O’neill, D.; Vadeyar, S.; Grant, J.; Ahmed, S.F.; Tolmie, J.; McCann, E.; Lam, W.; Smith, S.; et al. WT1 mutations in Meacham syndrome suggest a coelomic mesothelial origin of the cardiac and diaphragmatic malformations. *Am. J. Med. Genet.* **2007**, *143A*, 2312–2320. [[CrossRef](#)]
41. Zamora, M.; Männer, J.; Ruiz-Lozano, P. Epicardium-derived progenitor cells require beta-catenin for coronary artery formation. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 18109–18114. [[CrossRef](#)]
42. Mahtab, E.A.F.; Vicente-Steij, R.; Hahurij, N.D.; Jongbloed, M.R.M.; Wisse, L.J.; DeRuiter, M.C.; Uhrin, P.; Zaujec, J.; Binder, B.R.; Schalij, M.J.; et al. Podoplanin Deficient Mice Show a RhoA-Related Hypoplasia of the Sinus Venosus Myocardium Including the Sinoatrial Node. *Dev. Dyn.* **2009**, *238*, 183–193. [[CrossRef](#)]

43. Arora, H.; Boulberdaa, M.; Qureshi, R.; Bitirim, V.; Gasser, A.; Messaddeq, N.; Dolle, P.; Nebigil, C.G. Prokineticin receptor-1 signaling promotes Epicardial to Mesenchymal Transition during heart development. *Sci. Rep.* **2016**, *6*, 25541. [[CrossRef](#)] [[PubMed](#)]
44. Bonet, F.; Añez, S.B.; Inácio, J.M.; Futschik, M.E.; Belo, J.A. CCBE1 Is Essential for Epicardial Function during Myocardium Development. *Int. J. Mol. Sci.* **2022**, *23*, 12642. [[CrossRef](#)] [[PubMed](#)]
45. Combs, M.D.; Braitsch, C.M.; Lange, A.W.; James, J.F.; Yutzey, K.E. NFATC1 promotes epicardium-derived cell invasion into myocardium. *Development* **2011**, *138*, 1747–1757. [[CrossRef](#)] [[PubMed](#)]
46. Zhang, H.; Huang, X.; Liu, K.; Tang, J.; He, L.; Pu, W.; Liu, Q.; Li, Y.; Tian, X.; Wang, Y.; et al. Fibroblasts in an endocardial fibroelastosis disease model mainly originate from mesenchymal derivatives of epicardium. *Cell Res.* **2017**, *27*, 1157–1177. [[CrossRef](#)] [[PubMed](#)]
47. Withana, M.; Uribe, C.; Gregoric, I.D.; Angelini, P. Low Origin of the Coronary Arteries and a Small Aortic Annulus. *Tex. Heart Inst. J.* **2019**, *46*, 222–224. [[CrossRef](#)] [[PubMed](#)]
48. Matthes, S.A.; Taffet, S.; Delmar, M. Plakophilin-2 and the migration, differentiation and transformation of cells derived from the epicardium of neonatal rat hearts. *Cell Commun. Adhes.* **2011**, *18*, 73–84. [[CrossRef](#)] [[PubMed](#)]
49. Yuan, P.; Cheedipudi, S.M.; Rouhi, L.; Fan, S.; Simon, L.; Zhao, Z.; Hong, K.; Gurha, P.; Marian, A.J. Single-Cell RNA Sequencing Uncovers Paracrine Functions of the Epicardial-Derived Cells in Arrhythmogenic Cardiomyopathy. *Circulation* **2021**, *143*, 2169–2187. [[CrossRef](#)]
50. Maione, A.S.; Stadiotti, I.; Pilato, C.A.; Perrucci, G.L.; Saverio, V.; Catto, V.; Vettor, G.; Casella, M.; Guarino, A.; Polvani, G.; et al. Excess TGF- β 1 Drives Cardiac Mesenchymal Stromal Cells to a Pro-Fibrotic Commitment in Arrhythmogenic Cardiomyopathy. *Int. J. Mol. Sci.* **2021**, *22*, 2673. [[CrossRef](#)]
51. Poelmann, R.E.; Gittenberger-de Groot, A.C.; Vicente-Steijn, R.; Wisse, L.J.; Bartelings, M.M.; Everts, S.; Hoppenbrouwers, T.; Kruihof, B.P.T.; Jensen, B.; de Bruin, P.W.; et al. Evolution and Development of Ventricular Septation in the Amniote Heart. *PLoS ONE* **2014**, *9*, e106569. [[CrossRef](#)]
52. Poelmann, R.E.; Jensen, B.; Bartelings, M.M.; Richardson, M.K.; Gittenberger-de Groot, A.C.; Nakanishi, T.; Markwald, R.R.; Baldwin, H.S.; Keller, B.B.; Srivastava, D.; et al. The Epicardium in Ventricular Septation During Evolution and Development. In *Etiology and Morphogenesis of Congenital Heart Disease: From Gene Function and Cellular Interaction to Morphology*; Springer: Tokyo, Japan, 2016; Volume 25. [[CrossRef](#)]
53. Kolditz, D.P.; Wijffels, M.C.E.F.; Blom, N.A.; van der Laarse, A.; Hahurij, N.D.; Lie-Venema, H.; Markwald, R.R.; Poelmann, R.E.; Schalij, M.J.; Gittenberger-de Groot, A.C. Epicardium-Derived Cells in Development of Annulus Fibrosis and Persistence of Accessory Pathways. *Circulation* **2008**, *117*, 1508–1517. [[CrossRef](#)] [[PubMed](#)]
54. Verheule, S.; Tuyls, E.; Gharaviri, A.; Hulsmans, S.; van Hunnik, A.; Kuiper, M.; Serroyen, J.; Zeemering, S.; Kuijpers, N.H.; Schotten, U. Loss of continuity in the thin epicardial layer because of endomyocardial fibrosis increases the complexity of atrial fibrillatory conduction. *Circ. Arrhythm. Electrophysiol.* **2013**, *6*, 202–211. [[CrossRef](#)] [[PubMed](#)]
55. Yamaguchi, Y.; Cavallero, S.; Patterson, M.; Shen, H.; Xu, J.; Kumar, S.R.; Sucof, H.M. Adipogenesis and epicardial adipose tissue: A novel fate of the epicardium induced by mesenchymal transformation and PPAR γ activation. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 2070–2075. [[CrossRef](#)] [[PubMed](#)]
56. van Rosendaal, A.R.; Smit, J.M.; El'Mahdiui, M.; van Rosendaal, P.J.; Leung, M.; Delgado, V.; Bax, J.J. Association between left atrial epicardial fat, left atrial volume, and the severity of atrial fibrillation. *Europace* **2022**, *24*, 1223–1228. [[CrossRef](#)] [[PubMed](#)]
57. Otsuka, N.; Okumura, Y.; Arai, M.; Kurokawa, S.; Nagashima, K.; Watanabe, R.; Wakamatsu, Y.; Yagyu, S.; Ohkubo, K.; Nakai, T.; et al. Effect of obesity and epicardial fat/fatty infiltration on electrical and structural remodeling associated with atrial fibrillation in a novel canine model of obesity and atrial fibrillation: A comparative study. *J. Cardiovasc. Electrophysiol.* **2021**, *32*, 889–899. [[CrossRef](#)]
58. Nakamori, S.; Nezafat, M.; Ngo, L.H.; Manning, W.J.; Nezafat, R. Left Atrial Epicardial Fat Volume Is Associated With Atrial Fibrillation: A Prospective Cardiovascular Magnetic Resonance 3D Dixon Study. *J. Am. Heart Assoc.* **2018**, *7*, e008232. [[CrossRef](#)] [[PubMed](#)]
59. Yorgun, H.; Canpolat, U.; Aytemir, K.; Hazırolan, T.; Şahiner, L.; Kaya, E.B.; Kabakci, G.; Tokgözoğlu, L.; Özer, N.; Oto, A. Association of epicardial and peri-atrial adiposity with the presence and severity of non-valvular atrial fibrillation. *Int. J. Cardiovasc. Imaging* **2015**, *31*, 649–657. [[CrossRef](#)]
60. Zhao, L.; Harrop, D.L.; Ng, A.C.T.; Wang, W.Y.S. Epicardial Adipose Tissue Is Associated with Left Atrial Dysfunction in People Without Obstructive Coronary Artery Disease or Atrial Fibrillation. *Can. J. Cardiol.* **2018**, *34*, 1019–1025. [[CrossRef](#)]
61. Bos, D.; Vernooij, M.W.; Shahzad, R.; Kavousi, M.; Hofman, A.; van Walsum, T.; Deckers, J.W.; Ikram, M.A.; Heeringa, J.; Franco, O.H.; et al. Epicardial Fat Volume and the Risk of Atrial Fibrillation in the General Population Free of Cardiovascular Disease. *JACC Cardiovasc. Imaging* **2017**, *10*, 1405–1407. [[CrossRef](#)]
62. Oba, K.; Maeda, M.; Maimaituxun, G.; Yamaguchi, S.; Arasaki, O.; Fukuda, D.; Yagi, S.; Hirata, Y.; Nishio, S.; Iwase, T.; et al. Effect of the Epicardial Adipose Tissue Volume on the Prevalence of Paroxysmal and Persistent Atrial Fibrillation. *Circ. J.* **2018**, *82*, 1778–1787. [[CrossRef](#)] [[PubMed](#)]
63. Eren, H.; Omar, M.B.; Kaya, Ü.; Öcal, L.; Yılmaz, M.F.; Akkan, S. Epicardial adipose tissue may predict new-onset atrial fibrillation in patients with ST-segment elevation myocardial infarction. *J. Cardiovasc. Med.* **2021**, *22*, 917–923. [[CrossRef](#)] [[PubMed](#)]

64. Eren, H.; Omar, M.B.; Öcal, L. Epicardial fat tissue may predict new-onset atrial fibrillation in patients with non-ST-segment elevation myocardial infarction. *Turk. Kardiyol. Dern. Ars.* **2021**, *49*, 430–438. [[CrossRef](#)] [[PubMed](#)]
65. Hasebe, H.; Yoshida, K.; Nogami, A.; Ieda, M. Difference in epicardial adipose tissue distribution between paroxysmal atrial fibrillation and coronary artery disease. *Heart Vessel.* **2020**, *35*, 1070–1078. [[CrossRef](#)]
66. Sevinc, D.; Pasaoglu, L.; Coskun, R.; Atci, N.; Alimli, A.; Ucar, O. Relationships between left atrial pericardial fat and permanent atrial fibrillation: Results of a case-control study. *Diagn. Interv. Imaging* **2016**, *97*, 307–313, Erratum in: *Diagn. Interv. Imaging* **2017**, *98*, 283. Ucar, O.. [[CrossRef](#)] [[PubMed](#)]
67. Kim, T.H.; Park, J.; Park, J.K.; Uhm, J.S.; Joung, B.; Lee, M.H.; Pak, H.N. Pericardial fat volume is associated with clinical recurrence after catheter ablation for persistent atrial fibrillation, but not paroxysmal atrial fibrillation: An analysis of over 600-patients. *Int. J. Cardiol.* **2014**, *176*, 841–846. [[CrossRef](#)] [[PubMed](#)]
68. Chao, T.F.; Hung, C.L.; Tsao, H.M.; Lin, Y.J.; Yun, C.H.; Lai, Y.H.; Chang, S.L.; Lo, L.W.; Hu, Y.F.; Tuan, T.C.; et al. Epicardial adipose tissue thickness and ablation outcome of atrial fibrillation. *PLoS ONE* **2013**, *8*, e74926. [[CrossRef](#)]
69. Drossos, G.; Koutsogiannidis, C.P.; Ananiadou, O.; Kapsas, G.; Ampatzidou, F.; Madesis, A.; Bimpas, K.; Palladas, P.; Karagounis, L. Pericardial fat is strongly associated with atrial fibrillation after coronary artery bypass graft surgery. *Eur. J. Cardiothorac. Surg.* **2014**, *46*, 1014–1020, discussion 1020. [[CrossRef](#)]
70. Guzel, F.B.; Altunoren, O.; Gunes, H.; Seyithanoglu, M.; Kerkutluoglu, M.; Sezal, D.T.; Gungor, O. The relation between epicardial fat tissue thickness and atrial fibrillation in hemodialysis patients. *Semin. Dial.* **2020**, *33*, 428–434. [[CrossRef](#)]
71. Chu, C.Y.; Lee, W.H.; Hsu, P.C.; Lee, M.K.; Lee, H.H.; Chiu, C.A.; Lin, T.H.; Lee, C.S.; Yen, H.W.; Voon, W.C.; et al. Association of Increased Epicardial Adipose Tissue Thickness with Adverse Cardiovascular Outcomes in Patients With Atrial Fibrillation. *Medicine* **2016**, *95*, e2874. [[CrossRef](#)]
72. Nalliah, C.J.; Bell, J.R.; Raaijmakers, A.J.A.; Waddell, H.M.; Wells, S.P.; Bernasocchi, G.B.; Montgomery, M.K.; Binny, S.; Watts, T.; Joshi, S.B.; et al. Epicardial Adipose Tissue Accumulation Confers Atrial Conduction Abnormality. *J. Am. Coll. Cardiol.* **2020**, *76*, 1197–1211. [[CrossRef](#)] [[PubMed](#)]
73. Friedman, D.J.; Wang, N.; Meigs, J.B.; Hoffmann, U.; Massaro, J.M.; Fox, C.S.; Magnani, J.W. Pericardial fat is associated with atrial conduction: The Framingham Heart Study. *J. Am. Heart Assoc.* **2014**, *3*, e000477. [[CrossRef](#)] [[PubMed](#)]
74. Abe, I.; Teshima, Y.; Kondo, H.; Kaku, H.; Kira, S.; Ikebe, Y.; Saito, S.; Fukui, A.; Shinohara, T.; Yufu, K.; et al. Association of fibrotic remodeling and cytokines/chemokines content in epicardial adipose tissue with atrial myocardial fibrosis in patients with atrial fibrillation. *Heart Rhythm.* **2018**, *15*, 1717–1727. [[CrossRef](#)] [[PubMed](#)]
75. Kira, S.; Abe, I.; Ishii, Y.; Miyoshi, M.; Oniki, T.; Arakane, M.; Daa, T.; Teshima, Y.; Yufu, K.; Shimada, T.; et al. Role of angiotensin-like protein 2 in atrial fibrosis induced by human epicardial adipose tissue: Analysis using an organo-culture system. *Heart Rhythm.* **2020**, *17*, 1591–1601. [[CrossRef](#)] [[PubMed](#)]
76. Wang, Q.; Xi, W.; Yin, L.; Wang, J.; Shen, H.; Gao, Y.; Min, J.; Zhang, Y.; Wang, Z. Human Epicardial Adipose Tissue cTGF Expression is an Independent Risk Factor for Atrial Fibrillation and Highly Associated with Atrial Fibrosis. *Sci. Rep.* **2018**, *8*, 3585. [[CrossRef](#)] [[PubMed](#)]
77. Suffee, N.; Moore-Morris, T.; Jagla, B.; Mougnot, N.; Dilanian, G.; Berthet, M.; Proukhnitzky, J.; Le Prince, P.; Tregouet, D.A.; Pucéat, M.; et al. Reactivation of the Epicardium at the Origin of Myocardial Fibro-Fatty Infiltration During the Atrial Cardiomyopathy. *Circ. Res.* **2020**, *126*, 1330–1342. [[CrossRef](#)] [[PubMed](#)]
78. Meulendijks, E.R.; Al-Shama, R.F.M.; Kawasaki, M.; Fabrizi, B.; Neefs, J.; Wesselink, R.; Ernault, A.C.; Piersma, S.; Pham, T.V.; Jimenez, C.R.; et al. Atrial epicardial adipose tissue abundantly secretes myeloperoxidase and activates atrial fibroblasts in patients with atrial fibrillation. *J. Transl. Med.* **2023**, *21*, 366. [[CrossRef](#)] [[PubMed](#)]
79. Kusayama, T.; Furusho, H.; Kashiwagi, H.; Kato, T.; Murai, H.; Usui, S.; Kaneko, S.; Takamura, M. Inflammation of left atrial epicardial adipose tissue is associated with paroxysmal atrial fibrillation. *J. Cardiol.* **2016**, *68*, 406–411. [[CrossRef](#)]
80. Liu, Q.; Zhang, F.; Yang, M.; Zhong, J. Increasing Level of Interleukin-1 β in Epicardial Adipose Tissue Is Associated with Persistent Atrial Fibrillation. *J. Interferon Cytokine Res.* **2020**, *40*, 64–69. [[CrossRef](#)]
81. Li, B.; Po, S.S.; Zhang, B.; Bai, F.; Li, J.; Qin, F.; Liu, N.; Sun, C.; Xiao, Y.; Tu, T.; et al. Metformin regulates adiponectin signalling in epicardial adipose tissue and reduces atrial fibrillation vulnerability. *J. Cell Mol. Med.* **2020**, *24*, 7751–7766. [[CrossRef](#)]
82. Girerd, N.; Scridon, A.; Bessière, F.; Chauveau, S.; Geloën, A.; Bousset, L.; Morel, E.; Chevalier, P. Periatrial epicardial fat is associated with markers of endothelial dysfunction in patients with atrial fibrillation. *PLoS ONE* **2013**, *8*, e77167. [[CrossRef](#)] [[PubMed](#)]
83. Shaihov-Teper, O.; Ram, E.; Ballan, N.; Brzezinski, R.Y.; Naftali-Shani, N.; Masoud, R.; Ziv, T.; Lewis, N.; Schary, Y.; Levin-Kotler, L.P.; et al. Extracellular Vesicles From Epicardial Fat Facilitate Atrial Fibrillation. *Circulation* **2021**, *143*, 2475–2493. [[CrossRef](#)] [[PubMed](#)]
84. Liu, L.; Luo, F.; Lei, K. Exosomes Containing LINC00636 Inhibit MAPK1 through the miR-450a-2-3p Overexpression in Human Pericardial Fluid and Improve Cardiac Fibrosis in Patients with Atrial Fibrillation. *Mediat. Inflamm.* **2021**, *2021*, 9960241. [[CrossRef](#)] [[PubMed](#)]
85. Shi, X.; Shao, X.; Liu, B.; Lv, M.; Pandey, P.; Guo, C.; Zhang, R.; Zhang, Y. Genome-wide screening of functional long noncoding RNAs in the epicardial adipose tissues of atrial fibrillation. *Biochim. Biophys. Acta Mol. Basis Dis.* **2020**, *1866*, 165757. [[CrossRef](#)] [[PubMed](#)]

86. Pokushalov, E.; Kozlov, B.; Romanov, A.; Strelnikov, A.; Bayramova, S.; Sergeevichev, D.; Bogachev-Prokophiev, A.; Zheleznev, S.; Shipulin, V.; Salakhutdinov, N.; et al. Botulinum toxin injection in epicardial fat pads can prevent recurrences of atrial fibrillation after cardiac surgery: Results of a randomized pilot study. *J. Am. Coll. Cardiol.* **2014**, *64*, 628–629. [[CrossRef](#)] [[PubMed](#)]
87. Cho, K.I.; Kim, B.J.; Cha, T.J.; Heo, J.H.; Kim, H.S.; Lee, J.W. Impact of duration and dosage of statin treatment and epicardial fat thickness on the recurrence of atrial fibrillation after electrical cardioversion. *Heart Vessel.* **2015**, *30*, 490–497. [[CrossRef](#)] [[PubMed](#)]
88. Carmona, R.; López-Sánchez, C.; Garcia-Martinez, V.; Garcia-López, V.; Muñoz-Chápuli, R.; Lozano-Velasco, E.; Franco, D. Novel Insights into the Molecular Mechanisms Governing Embryonic Epicardium Formation. *J. Cardiovasc. Dev. Dis.* **2023**, *10*, 440. [[CrossRef](#)]
89. Streef, T.J.; Groeneveld, E.J.; van Herwaarden, T.; Hjortnaes, J.; Goumans, M.J.; Smits, A.M. Single-cell analysis of human fetal epicardium reveals its cellular composition and identifies CRIP1 as a modulator of EMT. *Stem Cell Rep.* **2023**, *18*, 1421–1435. [[CrossRef](#)]
90. Tyser, R.C.V.; Ibarra-Soria, X.; McDole, K.; Arcot Jayaram, S.; Godwin, J.; van den Brand, T.A.H.; Miranda, A.M.A.; Scialdone, A.; Keller, P.J.; Marioni, J.C.; et al. Characterization of a common progenitor pool of the epicardium and myocardium. *Science* **2021**, *371*, eabb2986. [[CrossRef](#)]
91. Quijada, P.; Trembley, M.A.; Misra, A.; Myers, J.A.; Baker, C.D.; Pérez-Hernández, M.; Myers, J.R.; Dirx, R.A., Jr.; Cohen, E.D.; Delmar, M.; et al. Coordination of endothelial cell positioning and fate specification by the epicardium. *Nat. Commun.* **2021**, *12*, 4155. [[CrossRef](#)]
92. Meier, A.B.; Zawada, D.; De Angelis, M.T.; Martens, L.D.; Santamaria, G.; Zengerle, S.; Nowak-Imialek, M.; Kornherr, J.; Zhang, F.; Tian, Q.; et al. Epicardioid single-cell genomics uncovers principles of human epicardium biology in heart development and disease. *Nat. Biotechnol.* **2023**, *3*, 1–14. [[CrossRef](#)]
93. Zou, R.; Zhang, M.; Zou, Z.; Shi, W.; Tan, S.; Wang, C.; Xu, W.; Jin, J.; Milton, S.; Chen, Y.; et al. Single-cell transcriptomics reveals zinc and copper ions homeostasis in epicardial adipose tissue of heart failure. *Int. J. Biol. Sci.* **2023**, *19*, 4036–4051. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.