Chapter 11
The Potential of the Epicardium to Act as a Source of Lymphatic Cells

Linda Klotz and Paul Riley

Abstract The lymphatic vasculature is a blind-ended network, crucial for tissue fluid homeostasis, immune surveillance, and lipid adsorption from the gut, as well as being the main route of cancer metastasis. The homeobox transcription factor *Prox1* is essential for the development of the lymphatic vasculature and is also expressed in the heart, where it is required for cardiomyocyte structure and growth as well as the cardiac conduction system. The cardiac lymphatic vasculature constitutes a largely overlooked but arguably very important part of the lymphatic system, which may have direct effects on cardiac morphogenesis, function, and response to injury. This chapter addresses whether the epicardium might act as a source of cells contributing to the cardiac lymphatics, by providing an overview of epicardial development and reflecting the current developmental potential of epicardium-derived progenitor cells. Furthermore, the morphogenesis of the coronary blood vasculature and controversy surrounding the origin of coronary vessels are elaborated to give a better understanding of endothelial development in the context of the heart. Finally, the specification of lymphatic endothelial cells and the development of the systemic lymphatic vasculature are described according to historical, venous versus non-venous origin, debate and linked to current knowledge on the potential for an epicardial contribution to the cardiac lymphatics.

Keywords Lymphatic • Heart • Prox1 • Epicardium • Development • Coronary vessel • Vasculogenesis • Angiogenesis • Epicardium-derived progenitor cell • Epithelial-to-mesenchymal transition • Lymphatic endothelial cell • Cardiovascular • Inflammation

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Introduction

Heart development begins approximately at embryonic day 7.0 (E7.0) in murine embryos and can be subdivided into three stages: early, mid-gestation, and late maturation stages. During this time, key events such as primary and secondary heart field development (cardiac progenitor cell specification), morphogenesis of the linear heart tube, cardiac looping and maturation, and growth and septation of the chambers take place. Beyond birth, further growth and maturation of the myocardium and other cardiac structures ensue [1]. The formation of the epicardium takes place during mid-gestational heart development, along with myocardial growth, endocardial cushion formation, ventricular septation and trabeculation, as well as coronary and lymphatic vessel development [2–4]. This chapter will provide a brief overview of the development of the epicardium, the coronary blood vasculature, and the specification and development of the systemic and cardiac lymphatic vasculature, and also discuss the possibility of whether the epicardium may play a role in cardiac lymphatic development.

The Epicardium

The chambers of the mature vertebrate heart consist of three tissue layers: the myocardium and the endocardium and epicardium, which line the myocardium on the inside and outside, respectively. The epicardium comprises a single-cell epithelial sheet and is connected to the myocardium by subepicardial connective tissue. The epicardium undergoes epithelial-to-mesenchymal transition (EMT) during heart development and during postnatal repair and regeneration [5] to give rise to a population of so-called epicardium-derived progenitor cells (EPDCs). These EPDCs invade the myocardium and differentiate into various cell types, including cardiac fibroblasts, smooth muscle cells, pericytes, and cardiomyocytes, and may also play a role in Purkinje fiber development [6, 7]. There is an accumulation of evidence to indicate that the interactions between the epicardium and myocardium are crucial for correct differentiation and patterning of the heart. It has been shown that secondary cardiac defects, for example, thinning of the myocardium or interventricular septal defects, are consequences of genetic or surgical perturbation of epicardial development [8–18].

Development of the Epicardium

The epicardium and its derivatives were originally thought to develop from the myocardium until the late 1960s, when this theory was disproven [19]. The true origin of the epicardium was not discovered until decades later, when research in multiple vertebrate model organisms demonstrated an extracardiac source of cells

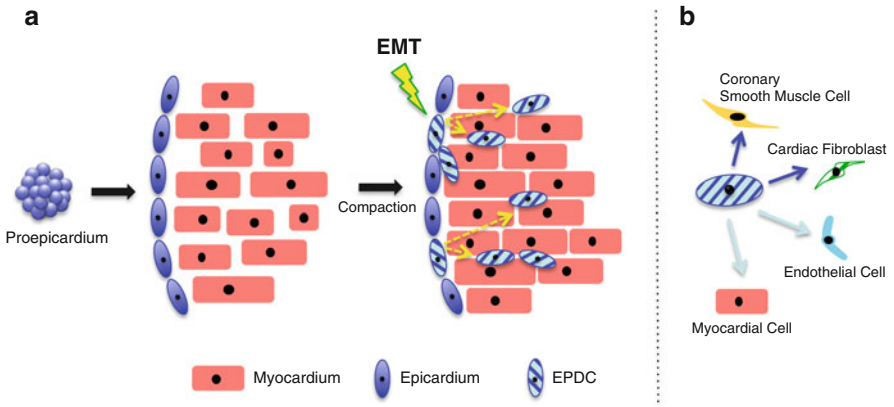

Fig. 11.1 (**a**) Proepicardial cells migrate towards the myocardium, where they attach to form a thin epithelial monolayer, the epicardium. A subset of epicardial cells undergoes epithelial-to-mesenchymal transition (EMT) to form epicardium-derived progenitor cells (EPDCs). EPDCs are induced to migrate into the subepicardial space and myocardium, leading to compaction of the myocardium, and subsequently differentiate into different cell types. (**b**) EPDCs can differentiate into coronary smooth muscle cells and interstitial cardiac and adventitial fibroblasts, with ongoing debate concerning their ability to differentiate into coronary endothelial or myocardial cells

giving rise to the epicardium: the proepicardium (PE), a cauliflower-shaped mass of coelomic cells which protrudes towards the pericardial cavity [20–22].

The proepicardium can first be visualized in mice around E8.5 [23] and is made up of a mesodermal cell population which protrudes immediately posterior to the sinoatrium in close proximity to the liver [20, 24–28]. The population is composed of at least two cell types: an external mesothelial epithelium and a mesenchymal core rich in extracellular matrix [28]. The proepicardium develops bilaterally symmetric in mice and zebra fish with two cell clusters that merge together to form a single proepicardium [22, 23, 27], whereas the chick proepicardium develops asymmetrically, with the right anlage appearing at HH-stage 14 [29] and the left anlage appearing at HH-stage 15/16. In chick embryos, only the right proepicardial anlage matures [30, 31]. Formation of the epicardial sheet requires the attachment of proepicardial cells to the naked myocardium. Proepicardial cells migrate towards the myocardial surface and subsequently flatten and spread to form a thin epithelial monolayer and a matrix-rich subepicardial space [20, 25, 26] (Fig. 11.1a). Starting in the atrioventricular groove, the epicardium grows to completely engulf the heart in a fixed spatiotemporal pattern as development progresses. The fate of the PE has been extensively studied in avian embryos using chick-quail chimeras and retroviral labeling techniques. The PE gives rise to most of the epicardium, with the exception of a small population near the outflow tract, which arises from the coelomic pericardial epithelium near the aortic sac [32]. Besides the primitive epicardium, the proepicardium also gives rise to blood endothelial cells; smooth muscle cells of the coronary blood vessels; perivascular, intermyocardial, and subendocardial fibroblasts; and a small number of endocardial cells [10, 33–37].

Epicardial Genes

There are a number of genes which have been associated with epicardial development, mostly based on expression patterns and mutant mouse phenotypes; however, their downstream targets are largely unknown. The *Wt1* gene encodes a zinc-finger protein which plays a role in tumor development [38] as well as kidney, urogenital, and epicardial development [15, 39]. *Wt1* is expressed in the primitive proepicardium-, epicardium-, and EPDCs, up until the point of differentiation, when its expression is downregulated [40]. Gata4, another zinc-finger protein, is expressed in the proepicardium and epicardium [41, 42], along with chicken Gata5 (cGATA5), which has been used to drive Cre-recombinase expression ectopically in the epicardium of transgenic mice [43]. GATA transcription factors also play a critical role during coronary artery formation [44]. Two T-box genes, *Tbx5* and *Tbx18*, are expressed during epicardial development [45]. The expression of *Tbx5* is transient and becomes lost after the epicardium has fully formed, while *Tbx18* is strongly expressed in the proepicardium and epicardium [30], although recent evidence suggests that *Tbx18* is dispensable for epicardial development [46]. Members of the forkhead transcription factor family, *FoxC1* and *FoxC2*, are expressed in a subset of cells in the proepicardium [47] and are also involved in kidney, cardiovascular, corneal, and cerebellar development and somitogenesis [48–51]. Interestingly, these transcription factors also play a role in lymphatic development [51, 52]. Another protein involved in both epicardial and lymphatic development is the transmembrane glycoprotein podoplanin [53, 54].

Deletion of key genes necessary for proepicardial and epicardial development have shown severe defects in cardiomyoblast proliferation as well as coronary vascular development [13–15, 42, 53, 55], although the effect on cardiac lymphatics has been entirely overlooked in this respect. Thus, the epicardium contributes to the development of key cardiac structures either by acting as a source of cell types or by providing signals, which will be described in more detail concerning coronary vasculogenesis, to direct the differentiation of other cells into distinct lineages. However, it is important to note that fate-mapping studies using epicardial marker genes for lineage trace analysis have proven to be difficult, as many of those genes, or the specific transgenic reporter/Cre constructs targeted to these loci, are also expressed in other tissues (earlier or later) during development (e.g., *Wt1* [56], *Tbx18* [57], *Gata5* [58]). This has made accurate lineage tracing, via Cre-lox strategies, of epicardial cell fate during development problematic. Furthermore, the timing and accuracy of Cre-mediated recombination depends not only on the expression level of the gene driving Cre activity but also on the sensitivity of the reporter construct itself [59]. Therefore, confirming the epicardial origin of a cell or tissue type requires extremely careful analysis, utilizing multiple approaches in parallel with lineage tracing to include accurate marker expression and conventional embryology (dye labeling, organ culture, etc.).

Epicardium-Derived Progenitor Cells

Once the PE cells have formed the primitive epicardium, a subset of epicardial epithelial cells undergo EMT to transform into a population of highly migratory, invasive cells called EPDCs [34], which then migrate into the subepicardial space [20] (Fig. 11.1a). It is currently still unclear whether all cells within the epicardium have the potential to undergo EMT and form EPDCs, selected on the basis of environmental cues or cell–cell interactions, or whether epicardial cells are heterogeneous and, therefore, only a subset are competent to undergo EMT [6, 60]. Recent evidence points towards a heterogeneous population in origin as well as function [61]. EMT happens throughout development to provide mesenchyme to developing embryonic structures, and in the heart it is observed at the atrioventricular junction, in the ventricular (but not atrial) epicardium, and at the junction between the ventricles and the outflow tract [62]. In mice, once EPDCs migrate into the myocardium, a thick, compact myocardial layer develops, which is more pronounced in the left ventricle [6]. The close reciprocal interactions between the myocardium and epicardium mean that any disturbance of epicardial outgrowth and EMT and migration of EPDCs leads to severe hypoplasia of the compact myocardium. This was shown via both mechanical inhibition of epicardial outgrowth [16] as well as in mouse mutants lacking key genes involved in these processes, including *PDGFRα*(alpha), *podoplanin*, and *Wt1* [53, 63–65]. It is generally accepted that EPDCs can differentiate into interstitial cardiac fibroblasts, adventitial fibroblasts, and coronary smooth muscle cells, confirmed by a series of seminal avian retroviral labeling and chick-quail chimera studies [33, 34, 37, 66, 67] (Fig. 11.1b) as well as, more recently, transgenic mouse lineage tracing studies [27, 33, 34, 37, 58, 68, 69]. There is some controversy and ongoing debate about the potential of EPDCs to differentiate into myocardial cells [70, 71] and coronary endothelium [6], of which the latter will be discussed in more detail in the next section.

Development of Coronary Blood Vessels

In the past three decades, there has been substantial progress in the understanding as to how systemic blood vessels develop as well as functional insight into the factors controlling their development [72]. Blood vessel development requires the tight coordination of cell proliferation, differentiation, migration, matrix adhesion, and cell–cell signaling [73]. It can be divided into vasculogenesis and angiogenesis. Vasculogenesis refers to the de novo formation of blood vessels via differentiation of angioblast or hemangioblast precursors, while angiogenesis denominates vascular sprouting or remodeling from existing vessels, commonly occurring throughout vascular development. It is thought that the primary capillary plexus is formed by vasculogenesis (e.g., the dorsal aorta and cardinal vein [74]) and later remodeled by angiogenesis [75, 76].

Although coronary blood vessels have been studied for over a century, comparatively little is known about the origin of cells and the underlying developmental programs of arguably one of the most important vascular networks. Simplistically, the heart pumps blood around the body through arteries, into smaller arterioles and even smaller capillary beds, which form vast plexi to exchange gases and metabolic products—only to be returned through venules and veins and then pumped to the lungs, where the blood is replenished with oxygen. The heart muscle continuously contracts to empty (diastole) and fill (systole) during the lifetime of adult mammals, and for this to work effectively, the heart is in constant need of its own supply of oxygen and nutrients from a fully functioning coronary vessel network. This requirement is consistent with mid-gestational development, when organ growth means that diffusion alone can no longer adequately support heart function.

Signaling Between the Epicardium and Coronary Vessels

Development of the coronary vasculature begins with the formation of a vascular network which is subsequently remodeled to give rise to the mature coronary tree [77–79]. This takes place in chick embryos from HH23 onwards [78] and in mouse embryos from E11.5 onwards [79, 80] and proceeds in four stages: migration of endothelial precursors, formation of an endothelial plexus, remodeling of the endothelial plexus, and growth and maturation of the coronary vessels [77]. While coronary vasculogenesis is largely complete by mid-gestation, the remodeling via angiogenesis and arteriogenesis continues throughout development and postnatally [81].

The development of the coronary vessels is closely associated with the development of the epicardium, which acts as a signaling center via multiple canonical pathways (e.g., retinoic acid (RA), fibroblast growth factor (FGF), hedgehog (HH), Wnt, and Notch) [60]. As mentioned previously, deletion of a number of genes necessary for epicardial development in the mouse has resulted in severe defects in cardiomyoblast proliferation and coronary vascular development [13–15, 42, 53, 55]. In *Fog2* (Friend-of Gata2) mutants, while the epicardium itself appears normal, there is a failure to undergo epicardial EMT, leading to severe defects in myocardial proliferation and coronary vessel development [44, 82]. Epicardial-derived FGF signals are also important for coronary vascular development, in addition to mediating myocardial growth [83], and have been shown to indirectly regulate coronary vascular plexus formation via HH signaling. Conditional knockouts of FGF9 and FGF-R1/FGF-R2 in mice results in delayed epicardial hedgehog expression and delayed coronary plexus formation due to the negative effect on downstream *Vegf-A*, *Vegf-B*, *Vegf-C*, and *Ang2* expression [80]. Two recent studies have suggested that the Notch pathway is also involved in the development of mouse epicardium and coronary vessels, as conditional mutants of Notch1 and its downstream effector, RBPJk, displayed severely reduced or abnormally dilated coronary vessels, respectively [84, 85].

Origin of Coronary Blood Endothelial Cells

For centuries the origin of coronary blood vessels was uncertain, and the assumption that they arise from endothelial sprouts originating in the aortic sinuses, myocardial sinusoids, and the sinus venosus was first made in the early twentieth century [86–88]. In the late 1980s, attempts to refute this assumption using descriptive data suggested that coronary arteries develop by ingrowth of a subepicardial endothelial plexus into the aorta [89–91]. Later quail-chick chimera studies on the one hand confirmed this hypothesis [67] and on the other hand demonstrated that cells of the coronary blood vessels come from a primarily extracardiac progenitor cell population, namely, the proepicardium [33–35, 92]. However, it is not clear whether quail angioblasts transplanted with the proepicardium differentiated there or whether they represented a migratory cell population (from a tissue such as the liver) that passed through the proepicardium en route to the subepicardium. Thus, there are two schools of thought dividing the field, with more recent mouse studies supporting both views.

Merki and colleagues reported no contribution of the proepicardial lineage to the coronary endothelium using cGata5-Cre transgenic mice [58]. A study by Red-Horse and colleagues demonstrated that coronary vessels arise from venous endothelial cells sprouting off the sinus venosus around E11.5, which dedifferentiate and are reprogrammed into arteries, capillaries, and veins [79]. However, this study did not exclude the possibility that endothelial cells may contribute to the coronary vasculature by coming from a distinct source (proepicardium, endocardium) or by arising as earlier progenitors elsewhere and then migrating into, or through, the sinus venosus [93]. This critique is further supported by findings from Katz and colleagues, who identified proepicardial markers *Scleraxis* and *Semaphorin 3D* which delineate previously uncharacterized proepicardial subcompartments. They demonstrated that the proepicardium itself is heterogeneous and has distinct subpopulations of cells, some of which can give rise to coronary endothelial cells via the sinus venosus [61].

Thus, the debate about the origin of coronary endothelial cells is still ongoing, with much remaining to be understood about the proepicardium as a cellular source of coronary endothelial cells.

As the development of the lymphatic vasculature occurs after the cardiovascular system and the first lymphatic vessels sprout from the cardinal vein, it is pertinent to acknowledge the close relationship between these two vascular networks. Understanding the connection between blood vessel and lymphatic vessel growth may lead to a greater insight into lymphatic development, particularly in organs where it has not been studied extensively.

Lymphatic Vessel Development

The lymphatic vasculature is a blind-ended network covering most tissues and organs of the body and is an essential component of vertebrate development and homeostasis. The lymphatic vasculature is crucial for tissue fluid regulation,

immune surveillance, and lipid adsorption from the gut and also serves as the main route for cancer metastasis. A number of human diseases are associated with reduced lymphatic vascular function or aberrant growth and development of lymphatic vessels. Problems in the lymphatic system can lead to pathologies such as lymphedema, delayed immune response, obesity, and hypertension [94].

The Historical Debate: Venous Versus Mesenchymal Origin?

The first published studies of the lymphatic vasculature date back to the mid-seventeenth century, when Gaspare Aselli, an Italian professor of anatomy and surgery, described the vessels he observed in a dog's abdomen as "milky veins." [95] Although further descriptive studies ensued, the origin of the lymphatic vessels remained unclear. In 1902, Florence Sabin proposed what remains to this day the most widely accepted model of lymphatic development. Following ink injection experiments performed on pig embryos, Sabin postulated that primary lymph sacs originate from endothelial cells which bud off from the veins [96]. This model suggests that the entire peripheral lymphatic vasculature develops by endothelial sprouting and remodeling from the primary lymph sacs, also known as the centrifugal theory. A few years later, an alternative, less popular model of lymphatic development was proposed by Huntington and McClure, who suggested that lymph sacs arise in the mesenchyme via distinct progenitor cells independently of veins, and later secondarily establish venous connections—the centripetal theory [97]. Although both models were proposed over a century ago, the discussion is still a current topic of interest, and more recent evidence has supported both the venous [98–101] as well as a distinct mesenchyme-derived lymphatic vasculature in various vertebrate model organisms [102–106].

Specification of Lymphatic Endothelial Cells

The specification of murine lymphatic endothelial cells (LECs) takes places during mid-gestation. The lymphatic vasculature begins to develop after the blood vasculature, and the cells that commit to a lymphatic identity originate within the dorsolateral walls of the cardinal veins. In the mouse at around embryonic day 9.5 (E9.5), a subset of COUP-TFII^{+} venous endothelial cells in the cardinal vein begin to express transcription factors *Sox18* and *Prox1*, and at that stage are classified as LEC progenitor cells [96, 98, 107–110]. *Sox18* binds directly to the proximal promoter of *Prox1* to activate its expression; however, *Sox18* expression is transient and as such only detected in LECs and newly forming vessels up to ~E14.5 [108]. Nevertheless, its role in developmental lymphangiogenesis is crucial as *Sox18*$^{-/-}$ embryos show a complete blockade of LEC differentiation from the cardinal vein [108]. This is consistent with *Prox1*-deficient embryos, which are devoid of lymphatic vessels due to a failure of LEC specification. Although sprouting from the veins is still observed, these *Prox1*-mutant cells have blood rather than lymphatic endothelial characteristics [109, 111].

Once specified in wild-type embryos, LECs subsequently delaminate from the walls of the anterior cardinal vein and migrate into the mesenchyme in a polarized manner between E10 and E11.5 to form primitive lymph sacs [111] (Fig. 11.2). Lymph sacs are the embryonic structures from which most of the lymphatic vasculature is eventually derived. The sprouting, proliferation, and migration of LECs away from the cardinal vein are regulated by Vegf-C through its receptor VEGFR-3, and it has been shown that sprouting of Prox1^{+} LECs in the cardinal vein is inhibited in Vegf-C$^{-/-}$ mutants [112, 113]. The lymphatic vasculature and blood vasculature are completely separated, with the exception of a few key connections where lymph is emptied back into the venous system at the junction of the thoracic duct and subclavian vein; in the renal, hepatic, and adrenal veins; and in the lymph nodes [114]. The blood-lymphatic separation is regulated by platelet aggregation at the junction sites between the cardinal vein and lymph sacs, induced by stimulation of the CLEC-2 receptor in platelets via podoplanin from LECs [115, 116]. The development and remodeling of the systemic lymphatic vasculature is complete by 2 weeks after birth [117]. Recent evidence has suggested that lymphatic vessels not only sprout from the cardinal vein but also from intersomitic veins [100] (Fig. 11.2), where the same pathways underlie lymphatic specification and migration. This broadens the scope of the developmental origin of lymphatic vessels, albeit still in support of the venous origin model.

Origins of Lymphatic Endothelial Cells

There are a number of different cell types, other than venous endothelial cells, which have been implicated in physically contributing to newly forming lymphatic vessels—interestingly, this only applies to lymphangiogenesis under pathological conditions or postnatally, rather than during embryonic development.

Pronounced neo-lymphangiogenesis has been found to be involved with the pathogenesis of organ rejection, psoriasis, airway inflammation, and rheumatoid arthritis [118–122], and NF-κB and LTα have recently been implicated in driving *Prox1* expression to initiate lymphangiogenesis during inflammation [123, 124]. However, the origin of cells contributing to newly formed vessels in pathological settings is ambiguous. Circulating precursors of LECs in mammals have been shown to differentiate from hematopoietic tissue-derived endothelial progenitors [125] which bear Syk- and Slp-76 antigens [126], from transdifferentiating leukocytes [106], or from transdifferentiating Cd11b^{+} macrophages [118, 127]. In addition, other studies have reported bone marrow-derived cells as a potential source, given their ability to acquire LEC antigens during lymphangiogenesis, both during normal homeostasis and under pathological conditions [128–130].

Recent findings have suggested that increasing lymphangiogenesis and stimulating lymphatic vessel function leads to a reduction in chronic skin inflammation [131, 132]; therefore understanding the origin of cells contributing to new vessels is crucial. In contrast, the origin of progenitor cells contributing to the cardiac lymphatic vasculature is entirely unknown. Furthermore, the role of the lymphatic

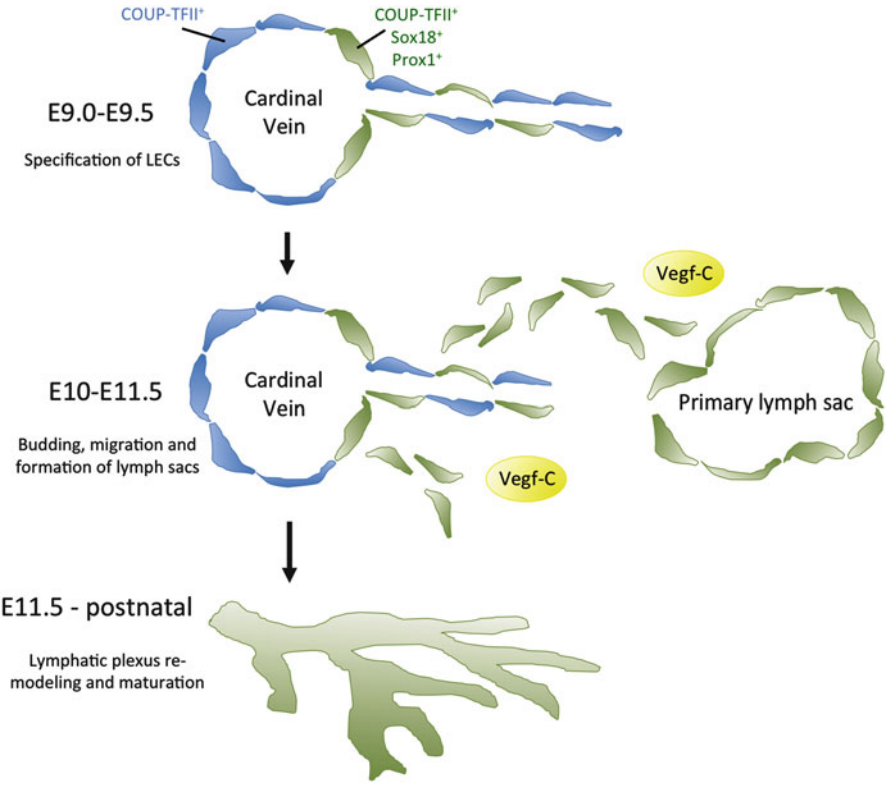

Fig. 11.2 The development of the murine lymphatic system. In mice, the LEC competence factor Sox18 starts to be expressed in a subpopulation of COUP-TFII$^+$ venous endothelial cells around E9.5. The commitment towards LEC differentiation is initiated upon Prox1 expression. Vegf-C signaling via Vegfr-3 induces Prox1-expressing cells to migrate away from the cardinal vein and intersomitic veins, by budding off to form the first primitive lymph sacs. Later, the whole lymphatic vascular network develops from these primitive lymph sacs and undergoes remodeling to form mature capillaries and vessels

system during cardiac inflammation is unknown, and in the context of adult cardiac injury (e.g., myocardial infarction), lymphatic vessel clearance of the inflammatory infiltrate may significantly improve cardiac outcome. A recent study demonstrated an increased remodeling of lymphatic vessels surrounding the infarct area; however, neither the cellular contribution and mechanisms underlying this response nor the potential functional benefits were examined [133].

The Cardiac Lymphatic Vasculature

The major focus thus far has been on the systemic lymphatic vasculature, while organ-based lymphatics have been largely overlooked. In particular, the lymphatic system in the heart has not been studied in great detail, and the mechanisms regulating cardiac

lymphatic development are yet to be described. Although recent publications describe the expression of lymphatic markers in developing murine and chick hearts [3, 4, 134], it is unclear where these LECs come from and whether other tissues may play a role in cardiac lymphatic development. The knowledge of the contribution of cardiac tissues will be beneficial to understanding the interplay between the heart and cardiac lymphatic vasculature, both during development and in disease.

Prox1 in the Heart

Prospero was originally isolated from *Drosophila* in 1991, where it was found to be fundamental for specifying neuronal fate [135, 136]. It was not until 1993 that *Prox1* (*prospero-related homeobox gene1*) was cloned in mice [137], and later its role in mammalian hematopoietic stem cell, retina, lens, and lymphatic development emerged [111, 138, 139]. The specific role in lymphatic development has been corroborated in other model organisms, such as chick [140], *Xenopus* [141], and zebra fish [141], revealing *Prox1* function to be highly conserved throughout evolution. Further studies have revealed that *Prox1* is the "master regulator" of lymphatic development [142], whose expression must be maintained within cells to retain a lymphatic identity and prevent them from being reprogrammed into blood endothelial cells (BECs) [143]. In this way, *Prox1* acts as a binary molecular switch, turning the BEC program "off" and the LEC program "on" [144].

In addition to being essential for the development of the lymphatic system, *Prox1* is also expressed in the heart [137] and has been implicated in maintaining cardiomyocyte structure and growth during heart development [145], and more recently in maintaining the adult cardiac conduction system [146]. Knocking out *Prox1* conditionally in cardiomyocytes has shown that *Prox1* is required for direct transcriptional regulation of the genes encoding structural proteins α-actinin, N-RAP, and zyxin, which collectively function to maintain association of the sarcomere [145]. Cardiomyocyte-specific *Prox1* knockout mice not only show abnormal heart development and defective sarcomere integrity compared to wild-type mice but also varying severities of edema, which is indicative of problems in the lymphatic system. Despite a wider understanding of the function of *Prox1* within the developing heart, the associated signaling pathways are still unknown.

The Role of the Epicardium During Cardiac Lymphatic Development

What is known thus far about the possible contribution of the proepicardium to coronary lymphatic vessels has largely arisen from chick-quail transplantation experiments. A study by Wilting and colleagues, in which a quail proepicardium was transplanted into

a chick host, demonstrated that avian cardiac lymphatics do not develop from the proepicardium [134]. Although they show donor-derived blood vessels in the chick host, the lymphatic vasculature is completely derived from the host itself, with the exception of one large lymphatic trunk at the base of the heart, attributed as a lympho-venous anastomosis, and thus possible homing of donor-derived venous endothelial cells into the Prox1^{+} lymphatic vessel. These results not only suggest that cardiac lymphatic vessels do not arise from the proepicardium or epicardium, but further imply that the cardiac lymphatic vessels do not arise from "local" venous endothelial cells (coronary veins) either, which themselves were donor-derived. As mentioned earlier, the current widely accepted dogma in lymphatic development is that all lymphatic vessels are derived from the cardinal vein and intersomitic veins [98, 100], which then sprout to give rise to all peripheral lymphatics. The findings from the chick-quail transplantation experiments would appear to support this theory.

It is significant to note that the aforementioned avian study is the first and only one of its kind investigating the contribution of the proepicardium to cardiac lymphatic vessels. As has been shown in the field of coronary vessel development, the discussion about the origin of endothelial cells in the heart is ongoing, specifically in reference to the variation seen between different model organisms and confounding species differences. Therefore, more investigation into this area of research needs to be performed to gain a deeper insight, especially given the relative paucity of appropriate mammalian models. A recent study showing different anatomical origins of vessels in the murine heart also emphasizes the possible heterogeneity of cells underlying cardiac lymphatic vessels [147]. There are a number of potential sources of progenitor cells that could contribute to the cardiac lymphatics. Although dermal lymphatics have been shown not to be derived from macrophages during development [148], macrophages are highly abundant during heart development [149] and therefore a candidate source for cardiac lymphatic development, which should be further investigated.

Despite transcription factors COUP-TFII, Sox18, and Prox1 being indispensable for lymphatic vascular development [150], there are a number of other pathways equally important for the correct development and remodeling of lymphatic vessels. Multiple studies have shown evidence for FGF signaling as being crucial for lymphatic development during embryogenesis and tumor vascularization and in ex vivo primary cell cultures. Shin and colleagues identified FGFR-3 as a direct target of Prox1 [151] and found it to be upregulated in LECs and lymphatic vessels throughout development. Further studies have shown that FGF-2-/FGFR-1-mediated lymphangiogenesis is dependent on Vegfr-3/Vegf-C signaling [152–155]. Retinoic acid has also been implicated in this pathway upstream of FGF receptor signaling [156]. In addition, classical Notch signaling has been shown to repress *Prox1* and *COUP-TFII* expression and the induction of LEC fate in vitro [157]. Interestingly, zebra fish and mouse models provide conflicting evidence suggesting that Notch signaling either plays a positive regulatory role or is not required at all for lymphangiogenesis [110, 158].

Conclusion

The development of the epicardium is a key process playing a central role during heart development. One factor complicating the study of epicardial progenitor cell origin and potential, and further confounded by current mouse models, is the lack of epicardial or EPDC-specific genes [159]. It is clear that the formation of the epicardium, as well as signaling between the epicardium and myocardium, is important for the correct development of the vessels and muscle of the embryonic heart, which in turn may also affect the morphogenesis and function of the cardiac lymphatic network. The aforementioned pathways involved in lymphatic development are also associated with epicardial development in conjunction with the coronary vasculature, and circumstantial evidence exists for a connection between lymphatic development and epicardial cell contribution to the heart. Therefore, although studies in the chick suggest that the epicardium does not directly contribute cells to the cardiac lymphatic vasculature, the emergence of new tools to study the epicardial lineage may assist in determining whether this is also true in mammals, including man. In addition, the adult epicardium can be induced to proliferate, migrate, and differentiate into cardiovascular derivatives following injury or in disease models [70, 160, 161], via the upregulation of an embryonic gene program. The possibility of an epicardial contribution to cardiac lymphatic vessels in a cardiovascular disease setting remains a tangible prospect, and one that is subject to ongoing investigation.

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