Chapter 11
Lymphatic Mechanoregulation in Development and Disease

Sandra A. Hemkemeyer, Claudia Carlantoni, and Maike Frye

Abstract Endothelial cells form the innermost layer of lymphatic and blood vessels and continuously interact with their luminal and tissue microenvironment. These interactions confer extracellular mechanical information, such as fluid shear stress, cellular stretch, and matrix stiffness, on the endothelium and are subsequently translated into intracellular biological responses. The impact of changes in fluid shear stress has been extensively studied in both lymphatic and blood endothelial cells. Recent studies suggest that the tissue microenvironment, which is established by the extracellular matrix, endothelial-associated mural cells, and the surrounding tissue, also fundamentally controls vascular development and disease.

In contrast to blood vessels, molecular mechanisms of lymphatic mechanoregulation via the tissue microenvironment are poorly understood. This review briefly compares what is known about the lymphatic and blood endothelial tissue microenvironment. We will further discuss how changes of the tissue microenvironment regulate lymphatic development and could contribute to dysregulation of lymphatic endothelial cells in disease. We aim to point out that a comprehensive analysis of tissue-regulated mechanisms could improve our understanding of lymphatic development and homeostasis and may eventually lead to the discovery of novel therapeutic approaches for lymphatic diseases associated with changes of the lymphatic-proximal microenvironment.

11.1 Introduction

In vertebrates, two complementary vessel networks form the vascular system and achieve organ homeostasis: the blood and the lymphatic vasculature. The *blood vasculature* is a circulatory system that transports oxygenated blood from the heart to peripheral tissues via arteries, while veins return oxygen-poor blood to the heart. In a hierarchical tree-like system, blood passes from large arteries to smaller

S. A. Hemkemeyer · C. Carlantoni · M. Frye (✉)
Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
e-mail: s.hemkemeyer@uke.de; c.carlantoni@uke.de; m.frye@uke.de

E. Papadimitriou, C. M. Mikelis (eds.), *Matrix Pathobiology and Angiogenesis*,
Biology of Extracellular Matrix 12, https://doi.org/10.1007/978-3-031-19616-4_11

arterioles and infiltrates tissues and organs through an extensive network of capillaries. The exchange of oxygen, delivery and recycling of nutrients and waste between blood and the tissue are achieved at the capillary level, which subsequently drains into venules and large veins. For re-oxygenation, blood is transported via the pulmonary artery to the lungs. In contrast, the *lymphatic vasculature* is a blind-ended, unidirectional vessel network that developed evolutionary to transport dietary fats from the intestine to the liver (Kampmeier 1969). Later, the lymphatic system acquired additional functions to drain excessive interstitial fluid and to transport immune cells to lymph nodes for immune surveillance (Stritt et al. 2021).

Endothelial cells (ECs) are essential vessel components and line the inner vessel wall of both vessel networks to form a dynamic barrier between the circulating blood or lymph on their luminal side and the surrounding tissue on their abluminal side. Through their specialized, button-like cell–cell contacts (button-like junctions) (Baluk et al. 2007), lymphatic capillaries (also referred to as initial lymphatics) collect fluid, macromolecules and cells from the interstitial tissues. The lymph is then transported via pre-collecting lymphatic vessels to the larger collecting lymphatic vessels and returns to the bloodstream via lymphovenous valves at the intersection of the jugular and subclavian veins (Jeltsch et al. 2003; Tammela and Alitalo 2010; Geng et al. 2016). Lymphatic collecting vessels are equipped with luminal lymphatic valves (LVs), which support pumping and ensure unidirectional lymphatic transport without reflux (Potente and Mäkinen 2017). In contrast to the lymphatic capillaries, the lymphatic collecting vessels form continuous zipper-like cell–cell contacts and are therefore characterized by a reduced absorption of fluid, macromolecules, and cells from their interstitial environment (Baluk et al. 2007).

Due to their unique functions, each vessel subtype is exposed to a variety of mechanical forces. These vessel subtypes are therefore equipped of specialized EC subtypes with unique properties and genetic profiles that enable them to fulfill their specific function (Potente and Mäkinen 2017). Not only does each vessel subtype exhibit a unique endothelial genetic profile, but endothelial properties also differ in a tissue-specific manner. As an example, specialized lymphatic ECs (LECs) are found in the Schlemm's canal vessels of the eyes (Petrova and Koh 2020), in the ascending vasa recta of the kidney (Kenig-Kozlovsky et al. 2018), and in meningeal lymphatic vessels in the brain (Aspelund et al. 2015; Louveau et al. 2015).

In addition, blood and lymphatic vessels are surrounded by unique tissue microenvironments with different mechanical and structural properties. ECs can be supported by various *extracellular matrix (ECM)* components, as well as by several *mural cell types*, like pericytes or smooth muscle cells (SMCs) (Gordon et al. 2020). The ECM is an essential part of the tissue environment and forms a complex three-dimensional scaffold consisting of the basement membrane (BM), which is mainly formed by the ECs themselves, and the interstitial matrix (IM), which fills the interstitial space between surrounding cells.

ECs recognize and respond to mechanical impacts, such as shear, stretch, and ECM stiffness, through their cell–cell contacts and cell–ECM adhesions, and translate physical stimuli into biological responses, in a process referred to as *mechanotransduction*. Shear mode and amplitude of fluid flow, as well as

composition and mechanical properties of the ECM, differ across the vascular tree, in development and disease.

While the impact of changes in fluid shear stress (FSS) is well studied in blood ECs (BECs) and LECs (reviewed in Baeyens et al. 2016; Bálint and Jakus 2021; Campinho et al. 2020), in this chapter we will focus on *changes of the lymphatic endothelial tissue microenvironment*. We will briefly compare what is known about LEC and BEC tissue microenvironments and further discuss how tissue microenvironment alterations could contribute to (dys)regulation of LECs in development and disease.

11.2 The Tissue Microenvironment of Endothelial Cells

11.2.1 The Endothelial Basement Membrane and Extracellular Matrix Components

During cell migration and sprouting in embryonic and postnatal (lymph)-angiogenesis, but also in pathological (lymph)angiogenic processes, the ECM surrounds individual ECs or EC clusters in three dimensions (3D). After vascular remodeling and maturation, ECs form the inner layer of lymphatic or blood vessels and adhere to the surrounding abluminal ECM environment in two dimensions (2D).

In the *established blood vasculature*, the blood vessel BM is mainly composed of laminin 411 (Thyboll et al. 2002; Stenzel et al. 2011) and 511 (Di Russo et al. 2017; Hallmann et al. 2005), collagen IV (Pöschl et al. 2004), fibronectin (Zhou et al. 2008; Van Obberghen-Schilling et al. 2011), and many other molecules, such as the proteoglycans perlecan (Zoeller et al. 2008; Lord et al. 2014; Douglass et al. 2015), agrin (Barber and Lieth 1997; Steiner et al. 2014), and nidogens (Bader et al. 2005). Besides collagen IV, additional collagens, such as collagens XVIII (Marneros et al. 2004), VIII (Sage and Iruela-Arispe 1990) and VI (Kuo et al. 1997; Groulx et al. 2011) have been identified to be part of the blood vessel BM. Genetic deletion or point mutations of genes encoding for these ECM components lead to severe blood vascular dysfunction (George et al. 1993; Thyboll et al. 2002; Van Obberghen-Schilling et al. 2011) and are often associated with embryonic or perinatal lethality (Costell et al. 1999; Pöschl et al. 2004; Bader et al. 2005; Coles et al. 2006). These findings support a pivotal function of BM components in blood vascular development. Additionally, it is not only important that ECM components are correctly expressed and assembled in the extracellular space, they also need to be remodeled and/or degraded to allow for functional blood vessel morphogenesis and homeostasis. This is achieved by matrix remodeling enzymes, such as matrix metalloproteinases (MMPs), reviewed in Sounni et al. (2011).

Although many studies have been performed focusing on the importance and contribution of the BM in the blood vasculature, less is known about the BM composition of the *established lymphatic vasculature*. Lymphatic vessel BM has

been shown to be composed of laminin 421 (Saito et al. 2009), collagen IV (Lutter et al. 2012), fibronectin (Ou et al. 2010; Podgrabinska et al. 2002), hyaluronan (El-Chemaly et al. 2009), and Emilin1 (Danussi et al. 2008). For example, the elastic microfibril-associated protein emilin1 is a component of the anchoring filaments in lymphatic vessels. *Emilin1* knockout mice display hyperplastic and disorganized lymphatic vessels with impaired drainage function, reduced number of anchoring filaments and dysfunctional cell-cell junctions (Danussi et al. 2008).

In contrast to the repertoire of remodeling MMPs expressed by BECs, reelin was initially considered a major lymphatic remodeling enzyme, which degrades fibronectin and laminin components (Lutter et al. 2012; Samama and Boehm 2005). However, important roles for MMPs during physiological and pathological lymphangiogenesis have also been discovered. For example, MMP14 has been shown to control lymphangiogenesis through regulation of lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) and vascular endothelial growth factor C (VEGFC) in mice (Wong et al. 2016). MMP2 blockage affects lymphatic tube formation in cell culture spheroids and ex vivo lymphatic ring assays (Ingvarsen et al. 2013). Similarly, inhibition of MMP2 and MMP9 has been associated with lower expression of VEGFC and vascular endothelial growth factor receptor 3 (VEGFR3) followed by impaired corneal lymphangiogenesis (Du et al. 2017). Taken together, these studies highlight a more complex contribution of MMPs in lymphatic vessel growth than previously thought.

To mediate adhesion to the tissue microenvironment, ECs are associated to the ECM through so-called focal adhesions, which are comprised of ECM binding proteins known as integrins (Gordon et al. 2020; Stupack and Cheresh 2004). Integrins bind to their specific ECM ligands and are intracellularly coupled to the cytoskeleton through actin binding proteins, including the mechanosensors vinculin and talin (Bays and Demali 2017; Yan and Schwartz 2018), to sense extracellular mechanical information and transduce them into the cell (Gordon et al. 2020). For example, fibronectin, which is not only an integral component of the lymphatic BM but also of the LV matrix, binds to endothelial integrin α_9 (Bazigou et al. 2009). EC-specific deletion of *Itgα9* or mice lacking the EIIIA domain of fibronectin show disorganized fibronectin network in the valve matrix resulting in dysplastic LV leaflets and retrograde lymph flow (Bazigou et al. 2009). Consequently, mechanosensing and mechanotransduction not only depend on ECM ligand availability, but also on the proper endothelial integrin repertoire.

Besides regulating adhesion and providing structural support for the endothelium, ECM components participate in the regulation of additional signaling pathways involved in blood vessel development and homeostasis through sequestration of chemokines or enhancement of chemokine activation. For example, VEGF signaling (Jakobsson et al. 2006; Stenzel et al. 2011; Cecchi et al. 2012), transforming growth factor β (TGFβ) signaling (Fontana et al. 2005), platelet-derived growth factor (PDGF) signaling (Lindblom et al. 2003), and angiopoietin signaling (Xu and Yu 2001; Xu et al. 2004; Chomel et al. 2009) have been identified to be regulated via the ECM. Similar to what has been shown for BECs, ECM components influence lymphatic signaling. Through ligation of integrin $\alpha_5ß_1$ and fibronectin, the

transactivation and phosphorylation of VEGFR3 are enhanced (Zhang et al. 2005). The collagen and calcium-binding EGF domain 1 (CCBE1) protein, which is expressed by mesenchymal cells close to the nascent lymphatics (Facucho-Oliveira et al. 2011), activates the cleavage of VEGFC to its active form, allowing for lymphatic sprouting (Jeltsch et al. 2014; Bos et al. 2011; Hogan et al. 2009).

Within the blood vasculature, relative amounts of ECM components have been shown to correlate with variable stiffness of the EC microenvironment. Arteries are stiffer (50–150 kPa, Kohn et al. 2015) than veins (3–50 kPa, Xue et al. 2017) because they need to withstand high blood pressure. In an ageing-related arterial stiffening mouse model, collagen I and III depositions have been shown to increase the incremental stiffness value of the arterial walls, indicating arterial stiffening in old mice (Fleenor et al. 2010).

In the developing embryo, collagen I is the predominant collagen type of the ECM and correlates with tissue stiffness (Majkut et al. 2013; Chen et al. 2012). Interestingly, analysis of collagen I deposition in embryonic day (E) 11 mouse embryos revealed substantial differences in collagen I support of nascent blood vascular versus lymphatic structures (Frye et al. 2018). Arteries and blood capillaries exhibited the highest collagen I density, while the cardinal vein (CV) and the migrating LECs showed moderate or low collagen I deposition, respectively (Fig. 11.1a, b). Decreasing collagen I density also correlated with decreasing local tissue stiffness experienced by ECs, suggesting that already during early vascular development the density of collagen I reflects an essential hierarchy of ECM stiffness required to fulfill different vascular functions.

However, it is important to note that the BM underlying most ECs is only a thin ECM layer (30–500 nm) (Liliensiek et al. 2009). Interestingly, cells can "feel" up to several micrometers deep into a soft substrate. Consistently, induction of EC network formation on compliant substrate (0.4 kPa) was prevented on very thin compliant substrates (<20 mm) as ECs sensed the stiffness of the underlying coverslip (Davidson et al. 2019). These findings suggest that the absolute in vivo stiffness of the ECM microenvironment experienced by ECs might rather be generated by several different surrounding tissue structures, including the ECM and, presumably, several layers of adjacent cells.

11.2.2 Mural Cell Support of the Endothelium

The specific tissue microenvironment of ECs is established not only by various ECM components, discussed in the previous paragraph, but also by surrounding cells. These cells can align the endothelium and provide a passive stiffness scaffold or are closely associated and actively regulating the endothelium, like mural cells.

Mural cells can be distinguished in pericytes and SMCs mostly depending on their morphology and location, although a rigorous distinction is not always possible. Pericytes are usually solitarily associated with small caliber vessels, but their relative frequency to ECs is highly variable (between 1:100 in skeletal muscle to 1:1

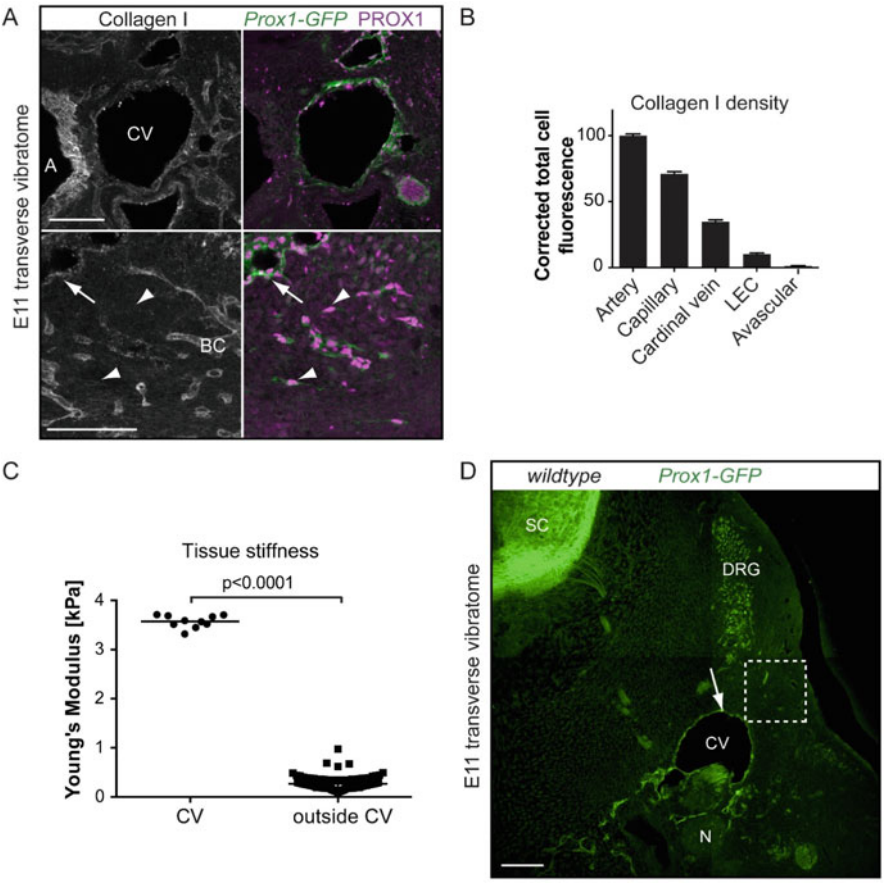

Fig. 11.1 Changes in matrix stiffness regulate early lymphatic development. (**a**) Immunofluorescence of transverse vibratome sections of E11 *Prox1-GFP* embryos using antibodies against PROX1 (magenta), GFP (green, Prox1 reporter), and collagen I (single channel images). Note, aorta (A) and blood capillaries (BC) show higher collagen I level compared to the CV (arrow) and migrating LECs (arrowheads). (**b**) Quantification of collagen I density in the respective vessel types and avascular tissue in E11 embryos. Data represent mean integrated density values of corrected total cell fluorescence ± s.e.m. (unpaired Student's t-test) quantified from $n = 10$ images taken from two embryos. (**c**) Ex vivo atomic force microscopy (AFM) measurements on transverse vibratome sections (image on the right) of E11 *Prox1-GFP* embryos. Young's Modulus (kPa) is a measure for the actual tissue stiffness. Horizontal lines represent mean ($n = 10$ measurements from one embryo (CV) and $n = 114$ measurements from three embryos (outside CV)). p value, unpaired Student's t-test. Measurements were done on the dorsal side of the CV (arrow) and the area of LEC migration (boxed area, outside CV). Prox1-GFP$^+$ spinal cord (SC), dorsal root ganglion (DRG), and nerve (N) were used for orientation. Scale bars: 100 μm. Modified from Frye M, et al. Nat Commun. 2018;9(1):1511 und licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/)

in the retina) (Armulik et al. 2005). Instead, SMCs can form multiple concentric layers around large caliber vessel types and can contract in order to facilitate the movement of fluids (Armulik et al. 2005; Gaengel et al. 2009). Both cell types derive from various cellular sources and through the combined action of hemodynamic forces and signaling induction, are recruited soon after the blood flow and circulation has been initiated (Shen and Mccloskey 2017). A well-studied signaling pathway is the platelet-derived growth factor subunit B (PDGF-B)/PDGF receptor (PDGFR) axis. Binding of PDGF-B, expressed by ECs, to the PDGFR, expressed on mural cells, activates mural cell proliferation and initiates their migration toward the vessels (Shen and Mccloskey 2017; Armulik et al. 2005). Global knockout mice for *Pdgfb* have been associated with microvascular aneurisms, loss of pericyte coverage, and embryonic lethality (Lindahl et al. 1997), while a more recent EC-specific knockout model exhibited increased retinal leakage in adult mice (Park et al. 2017).

In the lymphatic vasculature, mural cell recruitment starts from E 17.5 and proceeds after birth (Norrmén et al. 2009). PDGF signaling has been shown to be regulated downstream of forkhead box protein C2 (FOXC2) transcription factor (Petrova et al. 2004). Global knockout mice for *Foxc2* display upregulated *Pdgfb* expression, resulting in increased recruitment of pericyte/SMCs to the dermal lymphatic capillaries at E17.5, whereas control littermates did not show mural cell coverage of these vessels (Petrova et al. 2004). These studies highlight an essential role of mural cell recruitment to stabilize blood and lymphatic vessels during embryonic development and in vessel maintenance.

In the established vasculature, mural cell coverage and specific deposition of ECM components can distinguish different vessel subtypes by morphology and function. In the blood vasculature, capillaries are surrounded by pericytes and a continuous BM, although organ specific differences are possible; for example, in the liver sinusoids that exhibit discontinuous BM coverage (Potente and Mäkinen 2017). Large caliber vessels are instead surrounded by a continuous BM and an elastic lamina, consisting of numerous layers of SMCs, to withstand higher blood pressure (Potente and Mäkinen 2017). In the lymphatic vasculature, the evident morphological differences between initial and collecting lymphatics resemble the function of these two lymphatic vessel subtypes. Through a highly permeable barrier, which is devoid of a mural cell layer and a continuous BM, lymphatic capillaries drain fluids, macromolecules, and cells from the tissue. In contrast, collecting lymphatics transport the lymph back to the blood circulation and are therefore equipped with a continuous BM and SMCs that contract to allow the movement of the lymph (reviewed in Chen et al. 2014; Stritt et al. 2021).

Taken together, ECM composition and mural cell support can provide first hints about the local stiffness environment of ECs and their exposure to stretch. In the future, it will be necessary to analyze local in vivo stiffness experienced by ECs in more detail, to better understand the relationship between the different microenvironmental factors (ECM composition, mural cell support, and absolute tissue stiffness) and how the combined action of these factors could render ECs more susceptible to dysfunction. Particularly, the heterogeneity of the tissue

microenvironment between BECs and LECs might point toward the idea that selected mechanoregulatory processes and their threshold to malfunction may be essentially different in LECs. In the next paragraph, we will highlight recent findings on lymphatic mechanoregulation during development.

11.3 Mechanoregulation of Lymphatic Development

Changes of the microenvironment have been shown to fundamentally regulate cellular processes during development. For example, ECM stiffness regulates the differentiation of multipotent mesenchymal stem cells. Stiff matrices mimicking bone were found to be osteogenic, while soft matrices mimicking brain were neurogenic (Engler et al. 2006). Methylcellulose hydrogels, mimicking external constraints in the bone marrow, positively influenced megakaryocyte differentiation and proplatelet formation (Aguilar et al. 2016). Besides regulating cell fate and behavior at the single cell level, ECM stiffness can regulate tissue morphogenesis (Majkut et al. 2013; Poh et al. 2014).

Interestingly, fate decision of endothelial lineages is also dependent on the mechanical environment of the stem cell niche of vascular progenitor cells, as endothelial lineages prefer softer substrates (10 kilopascal (kPa)) and SMC lineages stiffer substrates (plastic, gigapascal (GPa) range) (Wong et al. 2019). Differences in ECM stiffness experienced by endothelial progenitor cells (EPCs) have been furthermore suggested to regulate arterial-venous differentiation in vitro (Xue et al. 2017). In contrast to EPCs that have been cultured on venous substrate stiffness (7 kPa), EPCs cultured on arterial substrate stiffness (128 kPa) showed an increase in expression of the arterial marker EphrinB2 (Zhang et al. 2005; Xue et al. 2017).

The *development of the mammalian lymphatic system* is initiated in the CV through trans-differentiation of a subpopulation of venous ECs around E9.5. These lymphatic endothelial progenitors express the transcription factor Prospero homeobox protein 1 (PROX1) (Yang et al. 2012; Wigle and Oliver 1999), which is activated through the SRY-Box transcription factor 18 (SOX18) (François et al. 2008; Yang et al. 2012; Srinivasan et al. 2007) and nuclear receptor subfamily 2 (NR2F2, also known as COUP-TFII) (Srinivasan et al. 2010).

Around E10.5, PROX1$^+$ LEC progenitors, expressing VEGFR3, start to delaminate from the CV and intersomitic vessels and migrate dorsolaterally toward a gradient of VEGFC to form the first lymphatic structures, the dorsal peripheral longitudinal lymphatic vessel (PLLV), and the ventral primordial thoracic duct (pTD) (commonly referred to as jugular lymph sacs (JLS)) (Yang et al. 2012; François et al. 2012; Hägerling et al. 2013).

The earliest evidence of lymphatic mechanoregulation via the tissue microenvironment has been demonstrated around E11, when lymphatic endothelial progenitors experience a decrease in ECM stiffness upon delamination from the CV (0.2 kPa outside the CV versus 4 kPa inside the CV) (Fig. 11.1c, d (Frye et al. 2018)). This decrease in matrix stiffness induces a GATA2-dependent transcriptional program,

which is required to form the first lymphatic vessels. Transcriptome analysis showed that LECs grown on a soft matrix (0.2 kPa vs. 25 kPa) exhibit increased GATA2 expression and a GATA2-dependent upregulation of genes involved in cell migration and lymphangiogenesis, including VEGFR3. Analysis of endothelial-specific *Gata2* deletion in mice demonstrated a cell-autonomous function of GATA2 in regulating LEC responsiveness to VEGFC, thereby controlling LEC migration and sprouting (Fig. 11.2). The study further compared the mechanosensitive transcriptional programs activated in LECs in response to different mechanical stimuli, such as increased matrix stiffness and oscillatory flow, and revealed that they appear remarkably different (Frye et al. 2018). Additionally, in contrast to increased GATA2 expression in LECs upon exposure to a soft matrix, GATA2 expression in BECs has been reported to increase in response to increased mechanical stimulus, such as matrix stiffening and induction of oscillatory flow (Mammoto et al. 2009; Kazenwadel et al. 2015). It has been described in several cell types, including ECs, that GATA2 interacts with other transcriptional regulators, including Etv2 (Shi et al. 2014) and Lmo2 (Coma et al. 2013), to form multimeric transcription complexes. An interesting question would be whether oscillatory flow- and soft matrix-induced differences in GATA2-mediated regulation of target genes and cellular responses can be explained by formation of different transcriptional complexes in different EC types.

In parallel to increased responsiveness to VEGFC that ensures efficient dorsolateral migration, proliferation is downregulated in LECs that experience a softer microenvironment. For example, when LECs were cultured on softer 2D substrates (0.2 kPa vs. 25 kPa), these cells also show a reduced proliferation parallel to an induction of VEGFR3 expression (Frye et al. 2018). Similarly, proliferation of BECs is reduced on soft 2D substrates but induced on rigid 2D substrates. Subconfluent human umbilical vein ECs (HUVECs) which were cultured on stiffer substrates (10 kPa vs. 1 kPa) increase vascular endothelial growth factor receptor 2 (VEGFR2) internalization and thus VEGFR2 activation (Lavalley et al. 2017). This phenomenon of reciprocal control of cell proliferation and migration has been described for other cell types (De Donatis et al. 2010). In the context of lymphatic development, it could be speculated that LECs are firstly subjected to an activation of their motility to populate the area of the JLS and secondly, once they have arrested migration, they induce a strong proliferative response to successfully expand the JLS.

A strong induction of proliferation indeed is achieved from E11.5 onward during lymphatic development, once JLS have formed and vessel expansion is induced (Planas-PAZ et al. 2012). Initially, an increase in interstitial pressure between E11.5 and E12 results in swelling of the interstitium, which leads to a stretching of the tissue and the ECM surrounding the JLS (Planas-PAZ et al. 2012), demonstrating again the importance of lymphatic mechanoregulation via the abluminal tissue microenvironment (Fig. 11.2). Stretching of LECs is evident by an elongation of the LECs between E11.5 and E12 and results in activation of VEGFR3 signaling and a transient strong increase in LEC proliferation, which was shown to depend on β_1 integrins (Planas-PAZ et al. 2012). Conversely, when interstitial fluid pressure decreases between E12.0 and E12.5, possibly due to efficient fluid drainage via

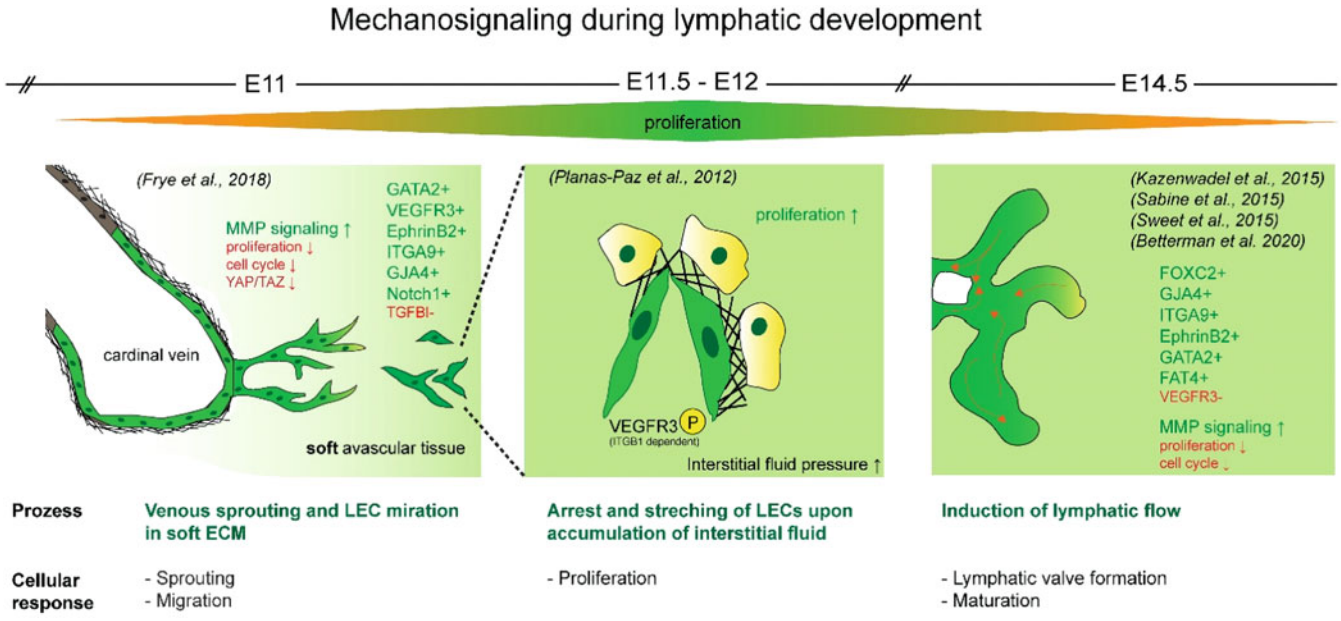

Fig. 11.2 Mechanosignaling during lymphatic development. Schematic of mechanoregulatory processes during lymphatic development. Initially, venous-derived LECs migrate out of the CV and are exposed to soft matrix, which induces GATA2-dependent increase in VEGFR3 expression and LEC migration. Next, LECs arrest and initiate proliferation in response to increased stretch, which is caused by an increase in interstitial fluid pressure. Last, initiation of lymphatic flow is necessary to induce lymphatic valve formation and maturation. Top regulated genes and signaling pathways (red, up; green, down) are indicated

the expanded lymphatic vasculature, the proliferation rate of LECs declines. In contrast to BECs, normal elongation of LECs in vitro is around 4%, whereas an increased elongation of 8% already leads to transcriptional induction of inflammatory cytokines, fibrotic markers, and lymphangiogenesis (Wang et al. 2017a). This could suggest that similar to EC type specific FSS set points (Baeyens et al. 2015), LECs have a lower stretch set point.

An indirect contribution of the immediate lymphatic microenvironment has also been identified during early lymphatic development. The CCBE1 protein is expressed by mesenchymal cells close to the nascent lymphatics (Facucho-Oliveira et al. 2011). CCBE1 does not have lymphangiogenic activity on its own, however it has been shown to activate a disintegrin and metalloproteinase with thrombospondin motifs 3 (ADAMTS3), which in turn cleaves VEGFC to its active form to allow for a proper VEGFC gradient and lymphatic sprouting, both in mouse (Jeltsch et al. 2014; Bos et al. 2011) and in zebrafish (Hogan et al. 2009; Wang et al. 2020).

Upon vessel expansion around E13.5, LECs of the pTD and the PLLV start to sprout toward the viscera and the superficial lymphatic plexus, respectively (Jafree et al. 2021). At the same time, non-venous LECs arise and start to coalesce to contribute to lymphatic vessel formation. In addition to the classical venous origin, it has been shown that LECs can also derive from other cell types, such as dermal blood capillary plexus (Pichol-Thievend et al. 2018), hemogenic endothelium (Stanczuk et al. 2015; Klotz et al. 2015), non-endothelial second heart field (Maruyama et al. 2019; Lioux et al. 2020), and paraxial mesoderm (Stone and Stainier 2019). These LECs appear as independent cell clusters that later fuse and incorporate into the nearby forming lymphatic vessels. This process is referred to as lymphvasculogenesis due to its similarity to the vasculogenesis process, where BECs differentiate from single precursors and then coalesce (Martinez-Corral et al. 2015). It seems likely that these additional LEC sources initially experience a different tissue microenvironment compared to the CV microenvironment with moderate stiffness (4 kPa). However, the potential impact of a differential microenvironment on non-venous derived LEC function has not been addressed yet and would be interesting to investigate in future studies.

Approximately at E14.5, an additional mechanical stimulus, the lymph FSS, is exerted on the LECs of the nascent lymphatic network. The onset of the intraluminal lymphatic flow has been shown to fundamentally contribute to the development of LVs (Fig. 11.2). LVs are predominantly formed at lymphatic branches, where the laminar flow pattern of the lymph is disturbed. At these valve initiation sites, oscillatory flow upregulates GATA2 that in turn maintains high PROX1 expression and transcriptionally activates FOXC2 transcription factor (Kazenwadel et al. 2015; Sabine et al. 2012). This transcriptional change activates the calcineurin/NFATC1 axis, connexin 37, and integrin α9 expression leading to valvulogenesis (Danussi et al. 2013; Sabine et al. 2012; Bazigou et al. 2009; Norrmén et al. 2009). Recently, an additional target of GATA2, the atypical cadherin FAT4, has been identified to control LEC polarity in response to flow and is required for lymphatic vessel morphogenesis, including valve formation (Betterman et al. 2020). By using mice that are deficient for the platelet-specific receptor C-type lectin-like receptor

2 (CLEC2), a complete block of lymphatic flow can be achieved as blood backfills the lymphatic network (Sweet et al. 2015). Similar to *Foxc2*-deficient vessels, *Clec2*-deficient lymphatic vessels are premature and excessive and fail to initiate valvulogenesis. Expression of PROX1, FOXC2, and VEGFR3 remains high in LECs of the mature valve but is downregulated in the mature lymphatic collecting vessels after LV formation (Norrmén et al. 2009). Presumably, disturbed flow patterns downstream of the LV maintain those expression patterns.

During postnatal lymphatic development, through secretion of PDGF-B, SMCs are recruited to lymphatic collecting vessels but not lymphatic capillaries (Sabine et al. 2012; Wang et al. 2017b). Another important phenomenon of collecting vessel maturation is the reduction of their diameter starting at E16.5 throughout postnatal stages (Norrmén et al. 2009). However, a potential direct impact of mechanical forces, such as matrix stiffening induced by mural cells or LEC constriction via the tissue microenvironment, has not been studied yet.

As development proceeds, collecting lymphatics and initial lymphatics remodel and mature. At the beginning of lymphatic development, all LECs are connected to each other through continuous zipper-like junctions (Yao et al. 2012). Remodeling of lymphatic capillary junctions to discontinuous button-like junctions increased from only 6% at E17.5 to 35% at birth, 50% at postnatal day (P)7 and 90% at P28 (Yao et al. 2012). This may be caused by mechanical forces generated through transmural lymph flow, which has been shown to induce delocalization and downregulation of vascular endothelial cadherin (VE-cadherin) and PECAM-1 in in vitro experiments (Miteva et al. 2010).

In addition to the mouse model system, *lymphatic development* has been extensively studied in *zebrafish*. Similar to mammals, in zebrafish LECs are distinguished from the rest of the endothelium by PROX1 expression and lymphangiogenesis is highly dependent on VEGFC/VEGFR3 signaling (Yaniv et al. 2006; Van Impel et al. 2014; Dunworth et al. 2014; Küchler et al. 2006; Le Guen et al. 2014; Shin et al. 2016). However, in contrast to mouse lymphatic development, a bipotent progenitor cell division underlies LEC specification in the CV of the zebrafish trunk (Koltowska et al. 2015; Nicenboim et al. 2015). From around 36 hours post-fertilization (hpf), sprouting of LEC progenitors and venous ECs occurs simultaneously, in a process called secondary sprouting (Yaniv et al. 2006; Küchler et al. 2006). About half of the sprouts form venous intersegmental vessels, while the remaining become parachordal lymphatic progenitors (PLs) in the horizontal myoseptum at around 52 hpf (Hogan et al. 2009). Subsequently, the PLs migrate ventrally and dorsally from the myoseptum to form the thoracic duct and the dorsal longitudinal lymphatic vessel, respectively (Yaniv et al. 2006). The network is then completed with the connection of these two main trunk lymphatic vessels through lymphatic intersegmental vessels. As in mouse, other non-venous sources of LECs have been discovered in zebrafish, such as the ventral aorta lymphangioblasts that contribute to facial lymphatics (Eng et al. 2019).

Formation of the proper lymphatic vascular network is highly dependent on the precise regulation of cell proliferation, which is achieved by the mitogenic VEGFC/VEGFR3/ERK signaling. Downstream of VEGFC/VEGFR3/ERK signaling, the

RNA-helicase DDX21 ensures for proper RNA biogenesis and cell cycle progression (Koltowska et al. 2021). Furthermore, similar to the process of LEC progenitor delamination from the CV in mice, during secondary sprouting in zebrafish proliferation is decreased (Jerafi-Vider et al. 2021). The decrease in proliferation was shown to be regulated via a VEGFC/VEGFR3/ERK-controlled cell cycle arrest. However, if the LEC microenvironment also guides cell cycle dynamics in zebrafish remains to be uncovered.

The microenvironment also plays an important role to guide LEC migration in zebrafish. Multiple cellular sources have been shown to guide migrating LECs and secrete guiding cues, such as chemokines and growth factors; these include intersegmental arteries, neurons, and fibroblasts (Bussmann and Raz 2015; Cha et al. 2012; Wang et al. 2020). In addition, LECs migrate alongside notochord sheath cells, which have been found to secrete localized type II collagen (Col2α1) to support PL migration through cell-ECM guidance (Chaudhury et al. 2020). Defects in Col2α1 secretion result in impaired migration of PLs. Similarly, the ECM protein polydom (also called Svep1), a ligand for integrin $\alpha_9\beta_1$, is expressed by mesenchymal cells in intimate proximity of remodeling venous ECs and LECs in zebrafish and mice (Morooka et al. 2017; Karpanen et al. 2017). Zebrafish *polydom/svep1* mutants exhibit a decrease in secondary sprouting, which leads to an increased number of intersegmental arteries. Consequently, a reduced number of PLs in horizontal myoseptum fails to migrate dorsally or ventrally and from the TD (Karpanen et al. 2017). These studies underline the importance to investigate the microenvironment of the developing lymphatic system. Whether, in addition to the local presence of selected ECM proteins, specific mechanical forces, such as matrix stiffness or stretch, are also involved in zebrafish, remains to be studied.

Mechanosensing of FSS-induced extracellular mechanical information has been extensively studied. FSS-induced mechanosensory mechanisms that regulate gene expression and cellular function include the regulation of ion channels and endothelial junctional protein complexes (Bálint and Jakus 2021). For example, the Piezo-type mechanosensitive ion channel component 1 (PIEZO1) has been identified to mediate mechanotransduction in the development and maintenance of the LVs (Nonomura et al. 2018; Choi et al. 2019). Furthermore, the calcium release-activated calcium modulator 1 (ORAI1), a pore subunit of the calcium release-activated calcium (CRAC) channel, is activated upon FSS and mediates Ca^{2+}-influx in LECs (Choi et al. 2017b) and induces Kruppel like factor 2 (KLF2) and KLF4 upregulation in LECs to promote VEGFC expression (Choi et al. 2017a).

Once extracellular mechanical information of the tissue microenvironment has been sensed through integrins and the FA complexes in BECs, the intracellular signals are propagated to the actin cytoskeleton. For example, with increasing matrix stiffness (3 kPa, 12 kPa, and 1.5 MegoPa), actin cytoskeleton remodeling becomes more organized, with an increasing amount of actin stress fibers (Jannatbabaei et al. 2019). The actin cytoskeleton is connected to VE-cadherin via its intracellularly associated proteins β- and α-catenin (Wessel et al. 2014). Tension or strain-induced actin remodeling tightly controls assembly and disassembly of VE-cadherin-based junctions (Oldenburg and De Rooij 2014). Additionally, cytoskeletal pulling at the

VE-cadherin complex also recruits the tension sensor protein vinculin via α-catenin to reinforce endothelial junctions (Huveneers et al. 2012; Daneshjou et al. 2015).

In contrast to BECs, our knowledge of stiffness-induced mechanosensory mechanisms in LECs is limited. Besides a direct effect on endothelial junction stability, cytoskeletal mechanotransduction can result in structural modification of membrane-bound or cytoplasmic proteins and their subsequent shuttling to the nucleus. For example, an important class of nuclear shuttling proteins consists of Yes-associated protein (YAP) and WW Domain-Containing Transcription Regulator Protein 1 (WWTR1/TAZ), which are downstream effectors of the Hippo pathway (Zhong et al. 2018). YAP/TAZ are shuttled to the nucleus in lymphatic ECs grown on stiff substrates (25 kPa vs. 0.2 kPa) and induce their target genes connective tissue growth factor *(CTGF)* and ankyrin repeat domain 1 (*ANKRD1)* (Frye et al. 2018). YAP/TAZ function has been extensively studied in the development of the blood (Neto et al. 2018; Sivaraj et al. 2020) and lymphatic vasculature (Cho et al. 2019; Cha et al. 2020; Grimm et al. 2019). Precisely how alterations in ECM stiffness might regulate those processes in LECs remains to be investigated.

Taken together, lymphatic development is guided via luminal (e.g. FSS) and abluminal mechanical forces, such as ECM composition, mural cell support, tissue stiffness and stretch capacity. Besides FSS-induced mechanotransduction, it is now highly relevant to investigate stiffness- and stretch-modulated LEC signaling pathways (including the identification of specific sensors and transducers), not only during lymphatic development but also during the maintenance of the established lymphatic system, as those pathways are likely to be dysregulated in a variety of disease conditions.

11.4 Mechano-Dysregulation of Lymphatic Endothelial Cells in Disease

Tissue remodeling and growth require ECM remodeling; however, aberrant ECM alterations have been associated with a variety of diseases, e.g. central nervous system (CNS) injury (Gaudet and Popovich 2014), tumor development and metastasis (Girigoswami et al. 2021; Nicolas-Boluda et al. 2021), lymphedema (Kistenev et al. 2019) or inflammatory bowel disease (Petrey and De La Motte 2017; Gordon et al. 2014). Uncontrolled remodeling of the ECM may lead to either an excessive degradation of ECM (Zhen and Cao 2014) or abnormal deposition and ECM stiffening (Frantz et al. 2010).

Comprehensive analyses of local stiffness changes experienced by LECs in diseased tissues have not been addressed. The temporal sequence of stiffness changes and LEC dysfunction and their interplay during disease progression are not understood. Here, we review selected diseases that are associated with lymphatic dysfunction and aim to point out possible hints that matrix alterations and

modulation of ECM stiffness do not only correlate with lymphatic dysfunction but might mutually define each other.

11.4.1 Lymphedema

Lymphedema is a chronic disease that can occur anywhere in the body, including extremities, face, thorax, and different body cavities. Lymphedema can be inherited (primary lymphedema), affecting 1 in 100,000 Americans (Smeltzer et al. 1985; Sleigh and Manna 2021), or caused by obstruction and injury of the lymphatic system (secondary lymphedema). Secondary lymphedema has a much higher incidence of 1 in 1000 people in developed countries, mainly due to malignant cancer treatment through lymph node (LN) dissection (Azhar et al. 2020). However, it is very likely that the incidence is underreported, especially in lower income countries (Torgbenu et al. 2020).

Several mutations in human genes have been identified to cause primary lymphedema, including *GATA2, SOX18, FOXC2, FLT4, PTPN14, PIEZO1* and *ITGA9*, affecting mainly LEC specification and lymphatic development (Oliver et al. 2020). For example, mutations in *FOXC2* cause Lymphedema-distichiasis (LD) syndrome with lymphatic vessels appearing normal but showing impaired lymphatic drainage due to valve dysfunction (Brice et al. 2002, Petrova et al. 2004, Brice et al. 2002). *Foxc2*-deficient mouse embryos and LD patients also display an ectopic mural cell and BM coverage (Petrova et al. 2004). Mutations in *FLT4* cause congenital bilateral lower limb lymphedema (Nonne-Milroy disease Karkkainen et al. 2004, Gordon et al. 2013) and loss-of-function mutations in *GATA2* lead to impaired development and maintenance of lymphovenous and lymphatic valves (Emberger Syndrome, Ostergaard et al. 2011, Kazenwadel et al. 2012).

Secondary lymphedema can additionally be caused by infection with the nematode *Wuchereria bancrofti*. The adult worm can obstruct lymphatic vessels and lymphatic transport, when lodging in the lymphatic system, and triggers inflammatory responses of the host (reviewed in Bennuru and Nutman 2009), which causes more than 60 million patients with lymphatic filariasis worldwide and about 25% of those patients suffering from lymphedema (Ramaiah and Ottesen 2014). A high prevalence of secondary lymphedema in the USA is also related to malignancy and tumor therapy. It has been described to accompany treatment routines of lymphoma, melanoma, urologic cancers and receives special attention for occurring after surgical and radiation therapy for breast cancer in women. The incidence of lymphedema after mastectomy ranges between 24% and 49%. Other studies report 4% to 28%, probably due to different measurement techniques and criteria (reviewed in Warren et al. 2007, Ly et al. 2017).

Due to ongoing fibrotic processes, it is a commonly accepted idea that lymphedema is associated with tissue stiffening, although lymphatic-proximal stiffness analysis has not been performed. Thus, it remains unclear how changes in ECM stiffness may affect LEC function and disease progression.

To study lymphedema in mice, several experimental lymphedema models, such as the mouse tail surgery model, the popliteal LN dissection and the related axillary LN dissection model, or an inducible transgenic lymphatic ablation model have been described (Ly et al. 2017). The mouse tail surgery model is the most commonly used lymphedema model. After carefully removing the 3–5 mm skin, the superficial and deep lymphatic vessels are ligated, while blood vessels are left intact. The resulting inflammation, lymphatic fluid stasis and vessel dilation, fibrosis and adipose deposition are mimicking the human post-surgical lymphedema. The observed edema increased interstitial pressure and fibrosis formation suggests stiffening of the tissue (Kashiwagi et al. 2011). Recently an advanced tail surgery model has been developed. One lymphatic collector is maintained intact, allowing the study of functional changes during disease progression in the intact vessel, while the common lymphedema phenotype can be observed in the disrupted lymphatic collector (Weiler et al. 2019).

In lymphedema, mainly collagen fibers are excessively deposited in both dermis and subcutaneous tissue (Gardenier et al. 2016). However, not much is known about the spatial organization of the collagen fiber network, although work by Wu et al. showed progressively less compacted and disorganized collagen, due to excessive fluid separating the collagen in the mouse tail model (Wu et al. 2011). Similarly, the development of limb lymphedema in patients with lymphadenectomy is convoyed by a thickening of the BM and an increase in collagen fibers (Mihara et al. 2012). The collagen increase was also confirmed in patients with stage II lymphedema by visualization of collagen fibers using second harmonic generation (Kistenev et al. 2019). More recently, a transgenic mouse model has been developed: a tamoxifen-inducible Cre-loxP system that expresses the human diphtheria toxin (DT) receptor under the control of the LEC-specific *Flt4* promoter. Injection of DT into any of the limbs results in a local ablation of lymphatics (Gardenier et al. 2016), resulting in a histological representation of the human disease by displaying comparable radiographical and clinical symptoms, including progressive dermal fibrosis and deposition of subcutaneous fibroadipose tissue. The latter has also been reported in the mouse tail surgery model, with an increase in fat thickness and subcutaneous fat deposition (Aschen et al. 2012). This is in agreement with several patient studies reporting that tissue swelling in lymphedema, can be caused through fluid stasis and fat deposition; for example, in lymphedemic limbs of breast cancer patients (Schaverien et al. 2018; Azhar et al. 2020).

Changes in ECM deposition are accompanied by another classic hallmark of lymphedema: chronic inflammation of the dermis and its underlying tissue. Lymphatic fluid stasis leads to an inflammatory response triggered by $CD4^+$ T cell infiltration into the surrounding tissue. Almost 70% of all inflammatory cells in lymphedema are $CD4^+$ T cells and their infiltration positively correlates with disease severity (Dayan et al. 2018; Ly et al. 2017). Interestingly, mice lacking all types of T cells or the $CD4^+$ subpopulation T cells fail to develop lymphedema in the tail surgery model. The infiltrating $CD4^+$ cells show a bias toward a T helper (Th) 2 response (Wynn 2008). Th2 cells are the main drivers of the inflammatory response by secreting pro-inflammatory/pro-fibrotic cytokines IL4 and IL13. Together with

upregulation of TGFβ1 signaling, this leads to fibrosis formation, which ultimately impairs lymphatic function (Dayan et al. 2018; Ly et al. 2017). Additionally, T cell-derived cytokines IL4, IL13, interferon gamma and TGFβ1 directly exhibit anti-lymphangiogenic function by decreasing LEC proliferation, migration, survival and responsiveness to VEGFC in human dermal LECs and in a mouse model of suture-induced corneal neovascularization (Savetsky et al. 2015). Besides $CD4^+$ T cells, other immune cells are also involved. Depletion of macrophages, for instance, led to increased $CD4^+$ T cell infiltration and Th2 differentiation and thus causing similar phenotypes with increased fibrosis and impaired lymphatic functions (Ghanta et al. 2015).

It can be speculated that, like chronic inflammation, severe changes in ECM deposition and stiffness are likely to directly contribute to lymphatic dysfunction and dysfunctional lymphangiogenesis or even prevent lymphatic re-growth in lymphedema. In agreement with this, in vitro experiments using human LECs demonstrate that decreasing matrix stiffness primes lymphatic tube formation, while increasing matrix prevented it (Alderfer et al. 2021). In human patients, stiffening of lymphedema tissue is seldomly assessed in a quantitative and objective way. The subjective view of the patients and medical staff (referring to tightening and stiffness) is the most common assessment (see, for example, Pekyavaş et al. 2014). The most common method for evaluating lymphedema severity is the circumference of the limb or volume determination, despite the knowledge that for some patients the affected limb softens due to treatment, but the circumference is not altered. Softening would indicate an improvement of disease (discussed and reviewed in Dayan et al. 2018, Hara and Mihara 2018). Additionally, macroscale indentation techniques, such as elastography and tonometry, have been employed to study tissue stiffness in lymphedema (Nowak and Kaczmarek 2018; Hara and Mihara 2018), but proper local stiffness values or parameters of early lymphedema stages are lacking. Although several studies try to identify risk parameters for lymphedema formation, like body mass index, age, and therapy approach, for example in women who underwent mastectomy for breast cancer treatment (Basta et al. 2017), it remains understudied whether stiffness increase, sensed by LECs, may serve as an early indicator of disease formation prior to clinical symptom development.

For secondary lymphedema, a potential LEC mechano-dysregulation has been proposed: due to surgical removal of a cancerous lymph node, the lymph flow between afferent (upstream) and efferent regions (downstream of the truncated LN) is disrupted. The lymph transport from the afferent region is abolished and leads to lymph accumulation in the lymphatic vessel, causing vessel dilation and a reduction in lymph drainage. It has been hypothesized that, similarly to the embryonic development, an increase in intestinal fluid and the swelling of the ECM is sensed by the LECs, resulting in an increase in β_1 integrin signaling, phosphorylation of VEGFR3, and elevated LEC proliferation with hyperplasia and further dilation of the lymphatic vessel (Planas-Paz and Lammert 2014).

Functional lymphatic drainage requires correctly organized LEC cell–cell junctions. It has been widely reported that LEC junctions are altered during inflammation. Mature button-like junctions can reversibly transform to zipper-like junctions in

inflammation (Yao et al. 2010). Zipper-like junctions reduce permeability and fluid uptake from the interstitial space is limited. This may be accompanied by reduced lymphatic flow (Zhou et al. 2010; Huggenberger et al. 2010; Cromer et al. 2015), although an initial increased flow has been reported in acute inflammation (Zhou et al. 2010). Both infections and inflammation are implicated in the development of secondary lymphedema (Yuan et al. 2019). Although little is known about how exactly the lymphatic system is altered in structure and function in lymphedema patients, Zhang et al. propose that LEC junction zippering may play a role in fluid retention and tissue swelling (Zhang et al. 2020). The authors suggest that the promotion of button formation could improve lymphatic drainage and subsequently reduce lymphedema. How changes of the tissue microenvironment could contribute to junctional alterations and how this might be addressed therapeutically needs to be further explored.

To date, lymphedema treatment is mainly limited to conservative therapies, such as manual drainage through physiotherapy and compression garments. Alternative treatments offer low-level laser therapy, stem cell therapy, and VEGFC treatment (Dayan et al. 2018; Oliver et al. 2020). However, clinical studies revealed that targeting these key regulators may increase the risk of metastasis and tumor recurrence in cancer patients (Skobe et al. 2001; Baker et al. 2010; Dayan et al. 2018).

Conclusively, identification and characterization of alternative lymphatic signaling pathways that are regulated via the tissue microenvironment could offer new possibilities to modulate and normalize lymphatic behavior in lymphedema disease conditions. At the same time, a comprehensive pre-symptomatic and symptomatic determination of ECM alterations in patients susceptible to lymphedema, such as cancer patients, could increase prevention of lymphedema development or improve treatment strategies.

11.4.2 Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is an umbrella term for multifactorial disorders of the digestive tract, leading to inflammation. Development and course of these diseases are not only determined via genetic susceptibility and immune dysregulation, but also via the microbial flora and environmental factors (reviewed in Lee and Chang 2021). Crohn's Disease (CD) and ulcerative colitis (UC) are two frequently observed types of IBD. While CD can potentially affect any area of the gastrointestinal tract and frequently causes transmural inflammation (all layers of the intestinal mucosa), UC predominantly affects the colon, and patients present with superficial ulcerations of the intestinal mucosa and submucosa (Eichele and Kharbanda 2017).

To study IBD-like diseases in mice, several mouse models have been developed. Genetically induced IBD can be observed in IL10 deficient mice that spontaneously develop colitis (Schwager and Detmar 2019), probably due to lack of the anti-inflammatory properties of IL10 (Kühn et al. 1993; Spencer et al. 2002), and in

TNFΔARE mice that develop ileitis as a consequence of deleted tumor necrosis factor (TNF) AU-rich elements (ARE) and dysregulated TNF biosynthesis (Kontoyiannis et al. 1999; Rehal and Von Der Weid 2017). Furthermore, murine IBD can be induced by administration of dextran sulfate sodium (DSS), at a concentration of 1–5% in the drinking water (Okayasu et al. 1990). DSS administration subsequently leads to a damage of the intestinal epithelium, thus compromising its barrier function so that luminal bacteria and associated antigens can enter the underlying tissue and release pro-inflammatory factors (Perše and Cerar 2012; Wirtz et al. 2007; Okayasu et al. 1990; Kiesler et al. 2015). If given for seven days, DSS induces an acute inflammation from which animals can recover, while for a chronic disease development, repeated administration cycles are required (Okayasu et al. 1990).

Although most studies have been conducted to investigate the contribution of immune cells, the breakdown of the epithelial barrier (Chidlow et al. 2007; Wei et al. 2020; Stürzl et al. 2021), or the role of the blood vasculature and VEGFA in IBD (Chidlow et al. 2011; Scaldaferri et al. 2009), it is important to note that the disease is also characterized by lymphatic vessel dilation, dysfunctional lymphangiogenesis and increased mesenteric lymphatic vessel leakage (Rehal et al. 2017), phenotypes also described for lymphedema.

Consistently, lymphatic vessel density is increased along with VEGFC expression in inflamed colons of IBD patients (D'Alessio et al. 2014). Furthermore, the study showed that in two murine disease models (DSS and IL10 deficient mice) systemic delivery of VEGFC can reduce disease severity by inducting proliferation to increase lymphatic vessel density. Consequently, lymphatic drainage was partly rescued with immune cells being mobilized from the inflamed intestine to the draining LNs. Together these findings suggest that functional lymphangiogenesis might be an important process for the resolution of intestinal inflammation (D'Alessio et al. 2014). In contrast to that, although DSS concentrations and animal age varied, another study showed that overexpression of VEGFC in older DSS treated mice led to a significant increase in clinical disease index, inflammatory edema, increased lymphatic vessel density and size, suggesting that at different IBD stages, lymphangiogenic processes may have pleiotropic effects (Wang et al. 2016). Similarly, dilated and leaking lymphatic vessels were observed in the ileal mucosa of TNFΔARE mice (Rehal et al. 2017).

BEC-derived MMPs have been shown to play fundamental roles in IBD (O'Shea and Smith 2014). Absence of the blood endothelial derived protease MT1-MMP from ECs impedes colitis progression, which is accompanied by limited deterioration of vascular perfusions and retained well-structured collagen fibers surrounding the colonic crypts (Esteban et al. 2020). Similarly, MT1-MMP expression and activity is elevated in ECs grown on stiffer glycan cross-linked ribose-collagen I substrates (0.5 kPa) compared to softer, more compliant collagen substrates (0.18 kPa) (Bordeleau et al. 2017). The angiogenic sprouting of EC spheroids was reduced in stiffer compared to the softer matrices. It can be speculated that similar signaling pathways might be involved in lymphatic dysregulation in IBD. This

would be in line with observations by D'Alessio et al. (2014) that lower density of lymphatic vessels might be linked to an increased risk of the recurrence of CD.

The lymphatic abnormalities in IBD are accompanied by changes in the ECM, which affect the tissue stiffness (as reported for lymphedema). A study in UC patients measuring the colonic tissue stiffness using a microelastomer, revealed a sixfold stiffness increase of unfixed UC strictures (16.7 kPa) in comparison to unaffected margins of the resected bowel (Johnson et al. 2013). The latter showed no difference compared to healthy intestine with 2.6 kPa and 2.9 kPa, respectively. Using a multi-scale indentation system, another study in seven CD and three UC patients conformingly observed an increase of steady-state modulus in inflamed tissue compared to unaffected areas, both for the colon (0.698 ± 0.463 kPa vs. 1.143 ± 0.488 kPa) and for the ileum (0.641 ± 0.342 kPa vs. 0.991 ± 0.379 kPa) (Stewart et al. 2018). Furthermore, they report an increase in *COL1A1* and *MMP-1* but no alterations of collagen IV and fibronectin content in inflamed tissue compared to unaffected areas. Notably, the study also highlights that colon stiffness (measured as effective total modulus) observed in the mouse, is two-fold higher than in unaffected human biopsies (8.493 ± 5.365 kPa versus 3.985 ± 2.656 kPa, respectively).

Interestingly, a softening of diseased colon tissue was observed in acute DSS at day (d)10 (6 kPa) and in ill IL10 deficient mice (3 kPa) compared to healthy wild-type mice, pre-symptomatic DSS at d4, and healthy IL10 deficient animals (11 kPa) (Shimshoni et al. 2021). This study employed AFM-based microscale stiffness analysis to determine local stiffness values. A softening of diseased colon tissue was further supported by deterioration of collagen, with regional ECM degradation and deposition, as well as a heterogenous ultrastructure (Shimshoni et al. 2021). For the first time, the authors further identify collagen XVIII and fibrillin 1 as biomarkers for a pre-symptomatic state, which still is void of clinical symptoms like body weight loss and endoscopic or histological phenotypes.

More in-depth research is needed to elucidate whether pathological ECM stiffness changes are fundamentally different in murine IBD-like disease compared to human IBD. Macroscale techniques like elastography (Maksuti et al. 2016) or tonography (Nowak and Kaczmarek 2018) to study tissue stiffness in lymphedemic and inflamed tissue analyze the general tissue stiffness or the entire vessel structure. However, they do not take into account cell-scale differences within the tissue, like changes of the EC-proximal tissue microenvironment. Microscale techniques like AFM (Frye et al. 2018) or 4D displacement microscopy (Vaeyens et al. 2020) could be better suited to answer these questions.

11.4.3 Tumor Microenvironment and Tumor Metastasis

Besides lymphedema and IBD, primary tumor development and tumor metastasis are known as lymphatic-associated processes (Oliver et al. 2020). The tumor tissue microenvironment is highly complex. Not only is it composed of different, often

dysregulated, cell types, such as ECs, fibroblasts, pericytes, or immune cells (reviewed in Labani-Motlagh et al. 2020), but the tumor microenvironment is often characterized by ECM stiffening (Trédan et al. 2007). For example, cancer cells can regulate collagen synthesis and in turn collagen may alter cancer cell behavior through integrin signaling (Levental et al. 2009). This observation is however not limited to collagen, but can be extended to fibronectin, laminin, and other ECM proteins (Baghban et al. 2020).

Solid tumors often induce the expansion of the surrounding lymphatic network, with matured lymphatic vessels being restricted to the tumor margin. As a consequence, the intestinal fluid accumulates in the tumor leading to an increase in interstitial pressure (Padera et al. 2016). Moreover, tumors are characterized by a remodeled ECM, including but not limited to collagen deposition and cross-linking (Northey et al. 2017) and stiffening, which strongly correlated with cancer progression and metastasis (reviewed in Emon et al. 2018).

For example, Wei and colleagues showed a positive correlation between an increase in collagen IV expression and human colorectal cancer progression (Wei et al. 2017). Another study, using HUVECs and bovine aortic ECs in spheroid assays showed that tumor angiogenesis (outgrowth, invasion, and vessel branching) is connected to an increased collagen I matrix cross-linking, which is linked to increased matrix stiffness, as assessed by measuring equilibrium compressive modulus. They were able to show that MT1-MMP activity is upregulated in stiffer collagen I matrix (100 mM ribose) compared to softer (0 nM ribose) matrix. The authors further showed that the increased matrix stiffness resulted in an impaired barrier function and mis-localized VE-Cadherin employing tunable polyacrylamide-based hydrogels to mimic stiff (10 kPa) and soft (0.2 kPa) environments (Bordeleau et al. 2017).

These stiffness changes can be aligned with studies that measured the stiffness of tumor tissue. For example, fibrotic colorectal cancer tissue had a median stiffness of 7.51 kPa, compared with about 0.936 kPa for healthy tissue when measured using a macroscale indentation device (Kawano et al. 2015). For mammary cancer (Levental et al. 2009), an almost ten-fold stiffer elastic modulus of around ~2 kPa has been reported compared to healthy tissue (~0.2 kPa), using unconfined compression analysis. How the alterations in stiffness may regulate or prevent (lymph)-angiogenesis is not fully understood, although the increase in interstitial fluid pressure is likely to cause compression of vessels, causing poor perfusion and resulting in hypoxia (Stylianopoulos et al. 2013). Additionally, it has been shown that BECs cultured within a stiffer 3D matrix sprout less compared to BECs cultured in softer 3D matrix (Trappmann et al. 2017), implicating that an unphysiologically stiff matrix could present a physical barrier for growing vessels.

LNs are the most common sites of tumor metastases and are crucial predictors of the cancer prognosis for the patient. The presence of tumor cells in the LN either reflects the cancer probability to metastasize, with the disease within the LN being inconsequential, or reflects the ability of the cancer cells in the lymph node to leave and spread the disease (Padera et al. 2016). Induction of VEGFC-mediated intratumor lymphangiogenesis at the location of the primary tumor can enable

metastatic breast cancer cells to enter the lymphatic vessels, allowing for increased metastasis in LNs and lung (Skobe et al. 2001). However, additional data have recently emerged. Using VEGFR3-blocking antibodies in mouse melanoma models, it has been shown that active VEGFC signaling enables a better immunotherapy response by recruiting naïve T cells through CCL21 induction, which are then locally activated (Fankhauser et al. 2017). In a glioblastoma mouse model, ectopic VEGFC expression led to an enhanced priming of $CD8^+$ T cells in the cervical LNs and migration of these T cells into the tumor, resulting in a rapid clearance of glioblastoma tumor (Song et al. 2020).

Taken together, lymphatic vessels play a crucial role not only in tumor development and metastasis, but also in tumor regression. It is likely that they respond to tumor ECM stiffness changes, and stiffening may prevent functional lymphatics to enter the tumor tissue. In parallel to the frequently discussed concept of blood vessel normalization (to facilitate chemotherapy) versus blood vessel regression (to starve the tumor) (Augustin and Koh 2022), similar questions should be applied to tumor lymphatic vessels. In addition to modulation of well-studied (lymph)angiogenic signaling pathways, normalization of the tumor microenvironment and stiffness (for example via "ECM softening") and, in particular, modulation of ECM stiffness-regulated lymphatic EC signaling pathways, might present a promising approach to tackle persisting hurdles in tumor therapy.

11.5 Summary

In contrast to blood endothelial mechanoregulation, the importance of lymphatic endothelial mechanoregulation via the tissue microenvironment has only recently been discovered. ECM composition and signaling capacity, as well as mural cell support of BECs, have been extensively studied. Identification of mechanoregulatory processes during lymphatic development and in vitro studies on LEC regulation via the matrix environment indicate that similar molecular mechanisms are likely to regulate LEC dysfunction in diseases associated with ECM alterations. Therefore, it appears particularly important now to extend our analysis with novel in vivo approaches of local, LEC-proximal tissue stiffness and stretch measurements to obtain a more holistic perspective. We believe it is necessary to analyze tissue stiffness and stretch capacity experienced by ECs, to understand (1) which microenvironmental factors (ECM composition, mural cell support, and absolute tissue stiffness) are key modulators of absolute tissue changes sensed by ECs, (2) how these factors mutually define each other, and (3) to identify thresholds of the combined action of these factors that render ECs more susceptible to dysfunction.

Acknowledgements We thank Dr. Kaska Koltowska for critically reading the part on lymphatic development in zebrafish. This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) grant FR4239/1-1 (MF).

Author Contributions MF conceptualized the manuscript. MF, SAH, and CC wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest The authors have no relevant financial or non-financial interests to disclose.

References

Aguilar A, Pertuy F, Eckly A, Strassel C, Collin D, Gachet C, Lanza F, Léon C (2016) Importance of environmental stiffness for megakaryocyte differentiation and proplatelet formation. Blood 128:2022–2032

Alderfer L, Russo E, Archilla A, Coe B, Hanjaya-Putra D (2021) Matrix stiffness primes lymphatic tube formation directed by vascular endothelial growth factor-C. FASEB J 35:e21498

Armulik A, Abramsson A, Betsholtz C (2005) Endothelial/pericyte interactions. Circ Res 97:512–523

Aschen S, Zampell JC, Elhadad S, Weitman E, De Brot AM, Mehrara BJ (2012) Regulation of adipogenesis by lymphatic fluid stasis: part II. Expression of adipose differentiation genes. Plast Reconstr Surg 129(4):838–847. https://doi.org/10.1097/PRS.0b013e3182450b47. PMID: 22456356; PMCID: PMC3445411

Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, Wiig H, Alitalo K (2015) A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med 212:991–999

Augustin HG, Koh GY (2022) Antiangiogenesis: vessel regression, vessel normalization, or both? Cancer Res 82:15

Azhar SH, Lim HY, Tan BK, Angeli V (2020) The unresolved pathophysiology of lymphedema. Front Physiol 11:137

Bader BL, Smyth N, Nedbal S, Miosge N, Baranowsky A, Mokkapati S, Murshed M, Nischt R (2005) Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice. Mol Cell Biol 25:6846–6856

Baeyens N, Nicoli S, Coon BG, Ross TD, Van Den Dries K, Han J, Lauridsen HM, Mejean CO, Eichmann A, Thomas JL, Humphrey JD, Schwartz MA (2015) Vascular remodeling is governed by a VEGFR3-dependent fluid shear stress set point. elife 4:e04645

Baeyens N, Bandyopadhyay C, Coon BG, Yun S, Schwartz MA (2016) Endothelial fluid shear stress sensing in vascular health and disease. J Clin Invest 126:821–828

Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, Kolahian S, Javaheri T, Zare P (2020) Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Sign 18:59

Baker A, KIM H, Semple JL, Dumont D, Shoichet M, Tobbia D, Johnston M (2010) Experimental assessment of pro-lymphangiogenic growth factors in the treatment of post-surgical lymphedema following lymphadenectomy. Breast Cancer Res 12:R70

Bálint L, Jakus Z (2021) Mechanosensation and mechanotransduction by lymphatic endothelial cells act as important regulators of lymphatic development and function. Int J Mol Sci 22:3955

Baluk P, Fuxe J, Hashizume H, Romano T, Lashnits E, Butz S, Vestweber D, Corada M, Molendini C, Dejana E, Mcdonald DM (2007) Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med 204:2349–2362

Barber AJ, Lieth E (1997) Agrin accumulates in the brain microvascular basal lamina during development of the blood-brain barrier. Dev Dyn 208:62–74

Basta MN, Wu LC, Kanchwala SK, Serletti JM, Tchou JC, Kovach SJ, Fosnot J, Fischer JP (2017) Reliable prediction of postmastectomy lymphedema: the risk assessment tool evaluating lymphedema. Am J Surg 213:1125–1133.e1

Bays JL, Demali KA (2017) Vinculin in cell-cell and cell-matrix adhesions. Cell Mol Life Sci 74: 2999–3009

Bazigou E, Xie S, Chen C, Weston A, Miura N, Sorokin L, Adams R, Muro AF, Sheppard D, Makinen T (2009) Integrin-alpha9 is required for fibronectin matrix assembly during lymphatic valve morphogenesis. Dev Cell 17:175–186

Bennuru S, Nutman TB (2009) Lymphatics in human lymphatic filariasis: in vitro models of parasite-induced lymphatic remodeling. Lymphat Res Biol 7:215–219

Betterman KL, Sutton DL, Secker GA, Kazenwadel J, Oszmiana A, Lim L, Miura N, Sorokin L, Hogan BM, Kahn ML, Mcneill H, Harvey NL (2020) Atypical cadherin FAT4 orchestrates lymphatic endothelial cell polarity in response to flow. J Clin Invest 130:3315–3328

Bordeleau F, Mason BN, Lollis EM, Mazzola M, Zanotelli MR, Somasegar S, Califano JP, Montague C, Lavalley DJ, Huynh J, Mencia-Trinchant N, Negrón Abril YL, Hassane DC, Bonassar LJ, Butcher JT, Weiss RS, Reinhart-King CA (2017) Matrix stiffening promotes a tumor vasculature phenotype. Proc Natl Acad Sci U S A 114:492–497

Bos FL, Caunt M, Peterson-Maduro J, Planas-Paz L, Kowalski J, Karpanen T, Van Impel A, Tong R, Ernst JA, Korving J, Van Es JH, Lammert E, Duckers HJ, Schulte-Merker S (2011) CCBE1 is essential for mammalian lymphatic vascular development and enhances the lymphangiogenic effect of vascular endothelial growth factor-C in vivo. Circ Res 109:486–491

Brice G, Mansour S, Bell R, Collin JR, Child AH, Brady AF, Sarfarazi M, Burnand KG, Jeffery S, Mortimer P, Murday VA (2002) Analysis of the phenotypic abnormalities in lymphoedema-distichiasis syndrome in 74 patients with FOXC2 mutations or linkage to 16q24. J Med Genet 39:478–483

Bussmann J, Raz E (2015) Chemokine-guided cell migration and motility in zebrafish development. EMBO J 34:1309–1318

Campinho P, Vilfan A, Vermot J (2020) Blood flow forces in shaping the vascular system: a focus on endothelial cell behavior. Front Physiol 11:552

Cecchi F, Pajalunga D, Fowler CA, Uren A, Rabe DC, Peruzzi B, Macdonald NJ, Blackman DK, Stahl SJ, Byrd RA, Bottaro DP (2012) Targeted disruption of heparan sulfate interaction with hepatocyte and vascular endothelial growth factors blocks normal and oncogenic signaling. Cancer Cell 22:250–262

Cha YR, Fujita M, Butler M, Isogai S, Kochhan E, Siekmann AF, Weinstein BM (2012) Chemokine signaling directs trunk lymphatic network formation along the preexisting blood vasculature. Dev Cell 22:824–836

Cha B, Ho Y-C, Geng X, Mahamud MR, Chen L, Kim Y, Choi D, Kim TH, Randolph GJ, Cao X, Chen H, Srinivasan RS (2020) YAP and TAZ maintain PROX1 expression in the developing lymphatic and lymphovenous valves in response to VEGF-C signaling. Development 147: dev195453

Chaudhury S, Okuda KS, Koltowska K, Lagendijk AK, Paterson S, Baillie GJ, Simons C, Smith KA, Hogan BM, Bower NI (2020) Localised Collagen2a1 secretion supports lymphatic endothelial cell migration in the zebrafish embryo. Development 147:dev190983

Chen X, Nadiarynkh O, Plotnikov S, Campagnola PJ (2012) Second harmonic generation microscopy for quantitative analysis of collagen fibrillar structure. Nat Protoc 7:654–669

Chen H, Griffin C, Xia L, Srinivasan RS (2014) Molecular and cellular mechanisms of lymphatic vascular maturation. Microvasc Res 96:16–22

Chidlow JH, Glawe JD, Pattillo CB, Pardue S, Zhang S, Kevil CG (2011) $VEGF_{164}$ isoform specific regulation of T-cell-dependent experimental colitis in mice. Inflamm Bowel Dis 17:1501–1512

Chidlow JH, Shukla D, Grisham MB, Kevil CG (2007) Pathogenic angiogenesis in IBD and experimental colitis: new ideas and therapeutic avenues. Am J Physiol Gastroin Liver Physiol 293:G5–G18

Cho H, Kim J, Ahn JH, Hong YK, Mäkinen T, Lim DS, Koh GY (2019) YAP and TAZ negatively regulate prox1 during developmental and pathologic lymphangiogenesis. Circ Res 124:225–242

Choi D, Park E, Jung E, Seong YJ, Hong M, Lee S, Burford J, Gyarmati G, Peti-Peterdi J, Srikanth S, Gwack Y, Koh CJ, Boriushkin E, Hamik A, Wong AK, Hong YK (2017a)

ORAI1 activates proliferation of lymphatic endothelial cells in response to laminar flow through krüppel-like factors 2 and 4. Circ Res 120:1426–1439
Choi D, Park E, Jung E, Seong YJ, Yoo J, Lee E, Hong M, Lee S, Ishida H, Burford J, Peti-Peterdi J, Adams RH, Srikanth S, Gwack Y, Chen CS, Vogel HJ, Koh CJ, Wong AK, Hong YK (2017b) Laminar flow downregulates notch activity to promote lymphatic sprouting. J Clin Invest 127: 1225–1240
Choi D, Park E, Jung E, Cha B, Lee S, Yu J, Kim PM, Lee S, Hong YJ, Koh CJ, Cho CW, Wu Y, Li Jeon N, Wong AK, SHIN L, Kumar SR, Bermejo-Moreno I, Srinivasan RS, Cho IT, Hong YK (2019) Piezo1 incorporates mechanical force signals into the genetic program that governs lymphatic valve development and maintenance. JCI Insight 4:e125068
Chomel C, Cazes A, Faye C, Bignon M, Gomez E, Ardidie-Robouant C, Barret A, Ricard-Blum S, Muller L, Germain S, Monnot C (2009) Interaction of the coiled-coil domain with glycosaminoglycans protects angiopoietin-like 4 from proteolysis and regulates its antiangiogenic activity. FASEB J 23:940–949
Coles EG, Gammill LS, Miner JH, Bronner-Fraser M (2006) Abnormalities in neural crest cell migration in laminin alpha5 mutant mice. Dev Biol 289:218–228
Coma S, Allard-Ratick M, Akino T, Van Meeteren LA, Mammoto A, Klagsbrun M (2013) GATA2 and Lmo2 control angiogenesis and lymphangiogenesis via direct transcriptional regulation of neuropilin-2. Angiogenesis 16:939–952
Costell M, Gustafsson E, Aszódi A, Mörgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fässler R (1999) Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol 147:1109–1122
Cromer W, Wang W, Zawieja SD, Von Der Weid P-Y, Newell-Rogers MK, Zawieja DC (2015) Colonic insult impairs lymph flow, increases cellular content of the lymph, alters local lymphatic microenvironment, and leads to sustained inflammation in the rat ileum. Inflamm Bowel Dis 21:1553–1563
D'Alessio S, Correale C, Tacconi C, Gandelli A, Pietrogrande G, Vetrano S, Genua M, Arena V, Spinelli A, Peyrin-Biroulet L, Fiocchi C, Danese S (2014) VEGF-C-dependent stimulation of lymphatic function ameliorates experimental inflammatory bowel disease. J Clin Invest 124: 3863–3878
Daneshjou N, Sieracki N, Van Nieuw Amerongen GP, Conway DE, Schwartz MA, Komarova YA, Malik AB (2015) Rac1 functions as a reversible tension modulator to stabilize VE-cadherin trans-interaction. J Cell Biol 208:23–32
Danussi C, Spessotto P, Petrucco A, Wassermann B, Sabatelli P, Montesi M, Doliana R, Bressan GM, Colombatti A (2008) Emilin1 deficiency causes structural and functional defects of lymphatic vasculature. Mol Cell Biol 28:4026–4039
Danussi C, Del Bel Belluz L, Pivetta E, Modica TM, Muro A, Wassermann B, Doliana R, Sabatelli P, Colombatti A, Spessotto P (2013) EMILIN1/α9β1 integrin interaction is crucial in lymphatic valve formation and maintenance. Mol Cell Biol 33:4381–4394
Davidson CD, Wang WY, Zaimi I, Jayco DKP, Baker BM (2019) Cell force-mediated matrix reorganization underlies multicellular network assembly. Sci Rep 9:12
Dayan JH, Ly CL, Kataru RP, Mehrara BJ (2018) Lymphedema: pathogenesis and novel therapies. Annu Rev Med 69:263–276
De Donatis A, Ranaldi F, Cirri P (2010) Reciprocal control of cell proliferation and migration. Cell Commun Sign 8:20
Di Russo J, Luik A-L, Yousif L, Budny S, Oberleithner H, Hofschröer V, Klingauf J, Van Bavel E, Bakker ENTP, Hellstrand P, Bhattachariya A, Albinsson S, Pincet F, Hallmann R, Sorokin LM (2017) Endothelial basement membrane laminin 511 is essential for shear stress response. EMBO J 36:183–201
Douglass S, Goyal A, Iozzo RV (2015) The role of perlecan and endorepellin in the control of tumor angiogenesis and endothelial cell autophagy. Connect Tissue Res 56:381–391
Du H-T, Du L-L, Tang X-L, Ge H-Y, Liu P (2017) Blockade of MMP-2 and MMP-9 inhibits corneal lymphangiogenesis. Graefes Arch Clin Exp Ophthalmol 255:1573–1579

Dunworth WP, Cardona-Costa J, Bozkulak EC, Kim J-D, Meadows S, Fischer JC, Wang Y, Cleaver O, Qyang Y, Ober EA, Jin S-W (2014) Bone morphogenetic protein 2 signaling negatively modulates lymphatic development in vertebrate embryos. Circ Res 114:56–66

Eichele DD, Kharbanda KK (2017) Dextran sodium sulfate colitis murine model: an indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. World J Gastroenterol 23:6016–6029

El-Chemaly S, Malide D, Zudaire E, Ikeda Y, Weinberg BA, Pacheco-Rodriguez G, Rosas IO, Aparicio M, Ren P, Macdonald SD, Wu HP, Nathan SD, Cuttitta F, Mccoy JP, Gochuico BR, Moss J (2009) Abnormal lymphangiogenesis in idiopathic pulmonary fibrosis with insights into cellular and molecular mechanisms. Proc Natl Acad Sci U S A 106:3958–3963

Emon B, Bauer J, Jain Y, Jung B, Saif T (2018) Biophysics of tumor microenvironment and cancer metastasis—a mini review. Comput Struct Biotechnol J 16:279–287

Eng TC, Chen W, Okuda KS, Misa JP, Padberg Y, Crosier KE, Crosier PS, Hall CJ, Schulte-Merker S, Hogan BM, Astin JW (2019) Zebrafish facial lymphatics develop through sequential addition of venous and non-venous progenitors. EMBO Rep 20:e47079

Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126:677–689

Esteban S, Clemente C, Koziol A, Gonzalo P, Rius C, Martínez F, Linares PM, Chaparro M, Urzainqui A, Andrés V, Seiki M, Gisbert JP, Arroyo AG (2020) Endothelial MT1-MMP targeting limits intussusceptive angiogenesis and colitis via TSP1/nitric oxide axis. EMBO Mol Med 12:e10862

Facucho-Oliveira J, Bento M, Belo JA (2011) Ccbe1 expression marks the cardiac and lymphatic progenitor lineages during early stages of mouse development. Int J Dev Biol 55:1007–1014

Fankhauser M, Broggi MAS, Potin L, Bordry N, Jeanbart L, Lund AW, Da Costa E, Hauert S, Rincon-Restrepo M, Tremblay C, Cabello E, Homicsko K, Michielin O, Hanahan D, Speiser DE, Swartz MA (2017) Tumor lymphangiogenesis promotes T cell infiltration and potentiates immunotherapy in melanoma. Sci Transl Med 9:eaal4712

Fleenor BS, Marshall KD, Durrant JR, Lesniewski LA, Seals DR (2010) Arterial stiffening with ageing is associated with transforming growth factor-β1-related changes in adventitial collagen: reversal by aerobic exercise. J Physiol 588:3971–3982

Fontana L, Chen Y, Prijatelj P, Sakai T, Fässler R, Sakai LY, Rifkin DB (2005) Fibronectin is required for integrin alphavbeta6-mediated activation of latent TGF-beta complexes containing LTBP-1. FASEB J 19:1798–1808

François M, Caprini A, Hosking B, Orsenigo F, Wilhelm D, Browne C, Paavonen K, Karnezis T, Shayan R, Downes M, Davidson T, Tutt D, Cheah KS, Stacker SA, Muscat GE, Achen MG, Dejana E, Koopman P (2008) Sox18 induces development of the lymphatic vasculature in mice. Nature 456:643–647

François M, Short K, Secker GA, Combes A, Schwarz Q, Davidson TL, Smyth I, Hong YK, Harvey NL, Koopman P (2012) Segmental territories along the cardinal veins generate lymph sacs via a ballooning mechanism during embryonic lymphangiogenesis in mice. Dev Biol 364:89–98

Frantz C, Stewart KM, Weaver VM (2010) The extracellular matrix at a glance. J Cell Sci 123: 4195–4200

Frye M, Taddei A, Dierkes C, Martinez-Corral I, Fielden M, Ortsäter H, Kazenwadel J, Calado DP, Ostergaard P, Salminen M, He L, Harvey NL, Kiefer F, Mäkinen T (2018) Matrix stiffness controls lymphatic vessel formation through regulation of a GATA2-dependent transcriptional program. Nat Commun 9:1511

Gaengel K, Genové G, Armulik A, Betsholtz C (2009) Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vasc Biol 29:630–638

Gardenier JC, Hespe GE, Kataru RP, Savetsky IL, Torrisi JS, Nores GDG, Dayan JJ, Chang D, Zampell J, Martínez-Corral I, Ortega S, Mehrara BJ (2016) Diphtheria toxin–mediated ablation of lymphatic endothelial cells results in progressive lymphedema. JCI Insight 1:e84095

Gaudet AD, Popovich PG (2014) Extracellular matrix regulation of inflammation in the healthy and injured spinal cord. Exp Neurol 258:24–34

Geng X, Cha B, Mahamud MR, Lim KC, Silasi-Mansat R, Uddin MKM, Miura N, Xia L, Simon AM, Engel JD, Chen H, Lupu F, Srinivasan RS (2016) Multiple mouse models of primary lymphedema exhibit distinct defects in lymphovenous valve development. Dev Biol 409:218–233

George EL, Georges-Labouesse EN, Patel-King RS, Rayburn H, Hynes RO (1993) Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. Development 119:1079–1091

Ghanta S, Cuzzone DA, Torrisi JS, Albano NJ, Joseph WJ, Savetsky IL, Gardenier JC, Chang D, Zampell JC, Mehrara BJ (2015) Regulation of inflammation and fibrosis by macrophages in lymphedema. Am J Physiol Heart Circ Physiol 308:H1065–H1077

Girigoswami K, Saini D, Girigoswami A (2021) Extracellular matrix remodeling and development of cancer. Stem Cell Rev Rep 17:739–747

Gordon K, Schulte D, Brice G, Simpson MA, Roukens MG, Van Impel A, Connell F, Kalidas K, Jeffery S, Mortimer PS, Mansour S, Schulte-Merker S, Ostergaard P (2013) Mutation in vascular endothelial growth factor-C, a ligand for vascular endothelial growth factor receptor-3, is associated with autosomal dominant milroy-like primary lymphedema. Circ Res 112:956–960

Gordon IO, Agrawal N, Goldblum JR, Fiocchi C, Rieder F (2014) Fibrosis in ulcerative colitis: mechanisms, features, and consequences of a neglected problem. Inflamm Bowel Dis 20:2198–2206

Gordon E, Schimmel L, Frye M (2020) The importance of mechanical forces for in vitro endothelial cell biology. Front Physiol 11:684

Goult BT, Yan J, Schwartz MA (2018) Talin as a mechanosensitive signaling hub. J Cell Biol 217:3776–3784

Grimm L, Nakajima H, Chaudhury S, Bower NI, Okuda KS, Cox AG, Harvey NL, Koltowska K, Mochizuki N, Hogan BM (2019) Yap1 promotes sprouting and proliferation of lymphatic progenitors downstream of Vegfc in the zebrafish trunk. eLife 8:e42881

Groulx JF, Gagné D, Benoit YD, Martel D, Basora N, Beaulieu JF (2011) Collagen VI is a basement membrane component that regulates epithelial cell-fibronectin interactions. Matrix Biol 30:195–206

Hägerling R, Pollmann C, Andreas M, Schmidt C, Nurmi H, Adams RH, Alitalo K, Andresen V, Schulte-Merker S, Kiefer F (2013) A novel multistep mechanism for initial lymphangiogenesis in mouse embryos based on ultramicroscopy. EMBO J 32:629–644

Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM (2005) Expression and function of laminins in the embryonic and mature vasculature. Physiol Rev 85:979–1000

Hara H, Mihara M (2018) Comparison of two methods, the sponge method and Young's modulus, for evaluating stiffness of skin or subcutaneous tissues in the extremities of patients with lymphedema: a pilot study. Lymphat Res Biol 16:464–470

Hogan BM, Bos FL, Bussmann J, Witte M, Chi NC, Duckers HJ, Schulte-Merker S (2009) Ccbe1 is required for embryonic lymphangiogenesis and venous sprouting. Nat Genet 41:396–398

Huggenberger R, Ullmann S, Proulx ST, Pytowski B, Alitalo K, Detmar M (2010) Stimulation of lymphangiogenesis via VEGFR-3 inhibits chronic skin inflammation. J Exp Med 207:2255–2269

Huveneers S, Oldenburg J, Spanjaard E, Van Der Krogt G, Grigoriev I, Akhmanova A, Rehmann H, De Rooij J (2012) Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. J Cell Biol 196:641–652

Ingvarsen S, Porse A, Erpicum C, Maertens L, Jürgensen HJ, Madsen DH, Melander MC, Gårdsvoll H, Høyer-Hansen G, Noel A, Holmbeck K, Engelholm LH, Behrendt N (2013) Targeting a single function of the multifunctional matrix metalloprotease MT1-MMP: impact on lymphangiogenesis*. J Biol Chem 288:10195–10204

Jafree DJ, Long DA, Scambler PJ, Ruhrberg C (2021) Mechanisms and cell lineages in lymphatic vascular development. Angiogenesis 24:271–288

Jakobsson L, Kreuger J, Holmborn K, Lundin L, Eriksson I, Kjellén L, Claesson-Welsh L (2006) Heparan sulfate in trans potentiates VEGFR-mediated angiogenesis. Dev Cell 10:625–634

Jannatbabaei A, Tafazzoli-Shadpour M, Seyedjafari E, Fatouraee N (2019) Cytoskeletal remodeling induced by substrate rigidity regulates rheological behaviors in endothelial cells. J Biomed Mater Res A 107:71–80

Jeltsch M, Tammela T, Alitalo K, Wilting J (2003) Genesis and pathogenesis of lymphatic vessels. Cell Tissue Res 314:69–84

Jeltsch M, Jha SK, Tvorogov D, Anisimov A, Leppänen VM, Holopainen T, Kivelä R, Ortega S, Kärpanen T, Alitalo K (2014) CCBE1 enhances lymphangiogenesis via A disintegrin and metalloprotease with thrombospondin motifs-3-mediated vascular endothelial growth factor-C activation. Circulation 129:1962–1971

Jerafi-Vider A, Bassi I, Moshe N, Tevet Y, Hen G, Splittstoesser D, Shin M, Lawson ND, Yaniv K (2021) VEGFC/FLT4-induced cell-cycle arrest mediates sprouting and differentiation of venous and lymphatic endothelial cells. Cell Rep 35:109255

Johnson LA, Rodansky ES, Sauder KL, Horowitz JC, Mih JD, Tschumperlin DJ, Higgins PD (2013) Matrix stiffness corresponding to strictured bowel induces a fibrogenic response in human colonic fibroblasts. Inflamm Bowel Dis 19:891–903

Kampmeier OF (1969) Evolution and comparative morphology of the lymphatic system. Thomas

Karkkainen MJ, Haiko P, Sainio K, Partanen J, Taipale J, Petrova TV, Jeltsch M, Jackson DG, Talikka M, Rauvala H, Betsholtz C, Alitalo K (2004) Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol 5:74–80

Karpanen T, Padberg Y, Van De Pavert SA, Dierkes C, Morooka N, Peterson-Maduro J, Van De Hoek G, Adrian M, Mochizuki N, Sekiguchi K, Kiefer F, Schulte D, Schulte-Merker S (2017) An evolutionarily conserved role for polydom/svep1 during lymphatic vessel formation. Circ Res 120:1263–1275

Kashiwagi S, Hosono K, Suzuki T, Takeda A, Uchinuma E, Majima M (2011) Role of COX-2 in lymphangiogenesis and restoration of lymphatic flow in secondary lymphedema. Lab Investig 91:1314–1325

Kawano S, Kojima M, Higuchi Y, Sugimoto M, Ikeda K, Sakuyama N, Takahashi S, Hayashi R, Ochiai A, Saito N (2015) Assessment of elasticity of colorectal cancer tissue, clinical utility, pathological and phenotypical relevance. Cancer Sci 106:1232–1239

Kazenwadel J, Secker GA, Liu YJ, Rosenfeld JA, Wildin RS, Cuellar-Rodriguez J, Hsu AP, Dyack S, Fernandez CV, Chong C-E, Babic M, Bardy PG, Shimamura A, Zhang MY, Walsh T, Holland SM, Hickstein DD, Horwitz MS, Hahn CN, Scott HS, Harvey NL (2012) Loss-of-function germline GATA2 mutations in patients with MDS/AML or MonoMAC syndrome and primary lymphedema reveal a key role for GATA2 in the lymphatic vasculature. Blood 119:1283–1291

Kazenwadel J, Betterman KL, Chong C-E, Stokes PH, Lee YK, Secker GA, Agalarov Y, Demir CS, Lawrence DM, Sutton DL, Tabruyn SP, Miura N, Salminen M, Petrova TV, Matthews JM, Hahn CN, Scott HS, Harvey NL (2015) GATA2 is required for lymphatic vessel valve development and maintenance. J Clin Invest 125:2979–2994

Kenig-Kozlovsky Y, Scott RP, Onay T, Carota IA, Thomson BR, GIL HJ, Ramirez V, Yamaguchi S, Tanna CE, Heinen S, Wu C, Stan RV, Klein JD, Sands JM, Oliver G, Quaggin SE (2018) Ascending vasa recta are angiopoietin/Tie2-dependent lymphatic-like vessels. J Am Soc Nephrol 29:1097–1107

Kiesler P, Fuss IJ, Strober W (2015) Experimental models of inflammatory bowel diseases. Cell Mol Gastroenterol Hepatol 1:154–170

Kistenev YV, Nikolaev VV, Kurochkina OS, Borisov AV, Vrazhnov DA, Sandykova EA (2019) Application of multiphoton imaging and machine learning to lymphedema tissue analysis. Biomed Opt Express 10:3353–3368

Klotz L, Norman S, Vieira JM, Masters M, Rohling M, Dubé KN, Bollini S, Matsuzaki F, Carr CA, Riley PR (2015) Cardiac lymphatics are heterogeneous in origin and respond to injury. Nature 522:62–67

Kohn JC, Lampi MC, Reinhart-King CA (2015) Age-related vascular stiffening: causes and consequences. Front Genet 6:112

Koltowska K, Lagendijk AK, Pichol-Thievend C, Fischer JC, Francois M, Ober EA, Yap AS, Hogan BM (2015) Vegfc regulates bipotential precursor division and prox1 expression to promote lymphatic identity in zebrafish. Cell Rep 13:1828–1841

Koltowska K, Okuda KS, Gloger M, Rondon-Galeano M, Mason E, Xuan J, Dudczig S, Chen H, Arnold H, Skoczylas R, Bower NI, Paterson S, Lagendijk AK, Baillie GJ, Leshchiner I, Simons C, Smith KA, Goessling W, Heath JK, Pearson RB, Sanij E, Schulte-Merker S, Hogan BM (2021) The RNA helicase Ddx21 controls Vegfc-driven developmental lymphangiogenesis by balancing endothelial cell ribosome biogenesis and p53 function. Nat Cell Biol 23:1136–1147

Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G (1999) Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated Immunopathologies. Immunity 10:387–398

Küchler AM, Gjini E, Peterson-Maduro J, Cancilla B, Wolburg H, Schulte-Merker S (2006) Development of the zebrafish lymphatic system requires Vegfc signaling. Curr Biol 16:1244–1248

Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274

Kuo HJ, Maslen CL, Keene DR, Glanville RW (1997) Type VI collagen anchors endothelial basement membranes by interacting with type IV collagen. J Biol Chem 272:26522–26529

Labani-Motlagh A, Ashja-Mahdavi M, Loskog A (2020) The tumor microenvironment: a milieu hindering and obstructing antitumor immune responses. Front Immunol 11:940

Lavalley DJ, Zanotelli MR, Bordeleau F, Wang W, Schwager SC, Reinhart-King CA (2017) Matrix stiffness enhances VEGFR-2 internalization, signaling, and proliferation in endothelial cells. Converg Sci Phys Oncologia 3:044001

Le Guen L, Karpanen T, Schulte D, Harris NC, Koltowska K, Roukens G, Bower NI, Van Impel A, Stacker SA, Achen MG, Schulte-Merker S, Hogan BM (2014) Ccbe1 regulates Vegfc-mediated induction of Vegfr3 signaling during embryonic lymphangiogenesis. Development 141:1239–1249

Lee M, Chang EB (2021) Inflammatory bowel diseases (IBD) and the microbiome-searching the crime scene for clues. Gastroenterology 160:524–537

Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, Yamauchi M, Gasser DL, Weaver VM (2009) Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139:891–906

Liliensiek SJ, Nealey P, Murphy CJ (2009) Characterization of endothelial basement membrane nanotopography in rhesus macaque as a guide for vessel tissue engineering. Tissue Eng Part A 15:2643–2651

Lindahl P, Johansson BR, Levéen P, Betsholtz C (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science 277:242–245

Lindblom P, Gerhardt H, Liebner S, Abramsson A, Enge M, Hellstrom M, Backstrom G, Fredriksson S, Landegren U, Nystrom HC, Bergstrom G, Dejana E, Ostman A, Lindahl P, Betsholtz C (2003) Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. Genes Dev 17:1835–1840

Lioux G, Liu X, Temiño S, Oxendine M, Ayala E, Ortega S, Kelly RG, Oliver G, Torres M (2020) A second heart field-derived Vasculogenic niche contributes to cardiac lymphatics. Dev Cell 52:350–363.e6

Lord MS, Chuang CY, Melrose J, Davies MJ, Iozzo RV, Whitelock JM (2014) The role of vascular-derived perlecan in modulating cell adhesion, proliferation and growth factor signaling. Matrix Biol 35:112–122

Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J (2015) Structural and functional features of central nervous system lymphatic vessels. Nature 523:337–341

Lutter S, Xie S, Tatin F, Makinen T (2012) Smooth muscle-endothelial cell communication activates Reelin signaling and regulates lymphatic vessel formation. J Cell Biol 197:837–849

Ly CL, Kataru RP, Mehrara BJ (2017) Inflammatory manifestations of lymphedema. Int J Mol Sci 18:171

Majkut S, Idema T, Swift J, Krieger C, Liu A, Discher DE (2013) Heart-specific stiffening in early embryos parallels matrix and myosin expression to optimize beating. Curr Biol 23:2434–2439

Maksuti E, Widman E, Larsson D, Urban MW, Larsson M, Bjällmark A (2016) Arterial stiffness estimation by shear wave elastography: validation in phantoms with mechanical testing. Ultrasound Med Biol 42(1):308–321. https://doi.org/10.1016/j.ultrasmedbio.2015.08.012. Epub 2015 Oct 9. PMID: 26454623

Mammoto A, Connor KM, Mammoto T, Yung CW, Huh D, Aderman CM, Mostoslavsky G, Smith LE, Ingber DE (2009) A mechanosensitive transcriptional mechanism that controls angiogenesis. Nature 457:1103–1108

Marneros AG, Keene DR, Hansen U, Fukai N, Moulton K, Goletz PL, Moiseyev G, Pawlyk BS, Halfter W, Dong S, Shibata M, LI T, Crouch RK, Bruckner P, Olsen BR (2004) Collagen XVIII/endostatin is essential for vision and retinal pigment epithelial function. EMBO J 23:89–99

Martinez-Corral I, Ulvmar MH, Stanczuk L, Tatin F, Kizhatil K, John SW, Alitalo K, Ortega S, Makinen T (2015) Nonvenous origin of dermal lymphatic vasculature. Circ Res 116:1649–1654

Maruyama K, Miyagawa-Tomita S, Mizukami K, Matsuzaki F, Kurihara H (2019) Isl1-expressing non-venous cell lineage contributes to cardiac lymphatic vessel development. Dev Biol 452:134–143

Mihara M, Hara H, Hayashi Y, Narushima M, Yamamoto T, Todokoro T, Iida T, Sawamoto N, Araki J, Kikuchi K, Murai N, Okitsu T, Kisu I, Koshima I (2012) Pathological steps of cancer-related lymphedema: histological changes in the collecting lymphatic vessels after lymphadenectomy. PLoS One 7:e41126

Miteva DO, Rutkowski JM, Dixon JB, Kilarski W, Shields JD, Swartz MA (2010) Transmural flow modulates cell and fluid transport functions of lymphatic endothelium. Circ Res 106:920–931

Morooka N, Futaki S, Sato-Nishiuchi R, Nishino M, Totani Y, Shimono C, Nakano I, Nakajima H, Mochizuki N, Sekiguchi K (2017) Polydom is an extracellular matrix protein involved in lymphatic vessel remodeling. Circ Res 120:1276–1288

Neto F, Klaus-Bergmann A, Ong YT, Alt S, Vion AC, Szymborska A, Carvalho JR, Hollfinger I, Bartels-Klein E, Franco CA, Potente M, Gerhardt H (2018) YAP and TAZ regulate adherens junction dynamics and endothelial cell distribution during vascular development. elife 7:e31037

Nicenboim J, Malkinson G, Lupo T, Asaf L, Sela Y, Mayseless O, Gibbs-Bar L, Senderovich N, Hashimshony T, Shin M, Jerafi-Vider A, Avraham-Davidi I, Krupalnik V, Hofi R, Almog G, Astin JW, Golani O, Ben-Dor S, Crosier PS, Herzog W, Lawson ND, Hanna JH, Yanai I, Yaniv K (2015) Lymphatic vessels arise from specialized angioblasts within a venous niche. Nature 522:56–61

Nicolas-Boluda A, Vaquero J, Vimeux L, Guilbert T, Barrin S, Kantari-Mimoun C, Ponzo M, Renault G, Deptula P, Pogoda K, Bucki R, Cascone I, Courty J, Fouassier L, Gazeau F, Donnadieu E (2021) Tumor stiffening reversion through collagen crosslinking inhibition improves T cell migration and anti-PD-1 treatment. eLife 10:e58688

Nonomura K, Lukacs V, Sweet DT, Goddard LM, Kanie A, Whitwam T, Ranade SS, Fujimori T, Kahn ML, Patapoutian A (2018) Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. Proc Natl Acad Sci U S A 115:12817–12822

Norrmén C, Ivanov KI, Cheng J, Zangger N, Delorenzi M, Jaquet M, Miura N, Puolakkainen P, Horsley V, Hu J, Augustin HG, Ylä-Herttuala S, Alitalo K, Petrova TV (2009) FOXC2 controls formation and maturation of lymphatic collecting vessels through cooperation with NFATc1. J Cell Biol 185:439–457

Northey JJ, Przybyla L, Weaver VM (2017) Tissue force programs cell fate and tumor aggression. Cancer Discov 7:1224–1237

Nowak J, Kaczmarek M (2018) Modelling deep tonometry of lymphedematous tissue. Phys Mesomech 21:6–14

Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology 98:694–702

Oldenburg J, De Rooij J (2014) Mechanical control of the endothelial barrier. Cell Tissue Res 355: 545–555

Oliver G, Kipnis J, Randolph GJ, Harvey NL (2020) The lymphatic vasculature in the 21st century: novel functional roles in homeostasis and disease. Cell 182:270–296

O'Shea NR, Smith AM (2014) Matrix metalloproteases role in bowel inflammation and inflammatory bowel disease: an up to date review. Inflamm Bowel Dis 20:2379–2393

Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, Dafou D, Kilo T, Smithson S, Lunt P, Murday VA, Hodgson S, Keenan R, Pilz DT, Martinez-Corral I, Makinen T, Mortimer PS, Jeffery S, Trembath RC, Mansour S (2011) Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet 43:929–931

Ou JJ, Wu F, Liang HJ (2010) Colorectal tumor derived fibronectin alternatively spliced EDA domain exserts lymphangiogenic effect on human lymphatic endothelial cells. Cancer Biol Ther 9:186–191

Padera TP, Meijer EF, Munn LL (2016) The lymphatic system in disease processes and cancer progression. Annu Rev Biomed Eng 18:125–158

Park DY, Lee J, Kim J, Kim K, Hong S, Han S, Kubota Y, Augustin HG, Ding L, Kim JW, Kim H, He Y, Adams RH, Koh GY (2017) Plastic roles of pericytes in the blood–retinal barrier. Nat Commun 8:15296

Pekyavaş N, Tunay VB, Akbayrak T, Kaya S, Karataş M (2014) Complex decongestive therapy and taping for patients with postmastectomy lymphedema: a randomized controlled study. Eur J Oncol Nurs 18:585–590

Perše M, Cerar A (2012) Dextran sodium sulphate colitis mouse model: traps and tricks. J Biomed Biotechnol 2012:718617

Petrey AC, De La Motte CA (2017) The extracellular matrix in IBD: a dynamic mediator of inflammation. Curr Opin Gastroenterol 33:234–238

Petrova TV, Koh GY (2020) Biological functions of lymphatic vessels. Science 369:eaax4063

Petrova TV, Karpanen T, Norrmén C, Mellor R, Tamakoshi T, Finegold D, Ferrell R, Kerjaschki D, Mortimer P, Ylä-Herttuala S, Miura N, Alitalo K (2004) Defective valves and abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis. Nat Med 10: 974–981

Pichol-Thievend C, Betterman KL, Liu X, Ma W, Skoczylas R, Lesieur E, Bos FL, Schulte D, Schulte-Merker S, Hogan BM, Oliver G, Harvey NL, Francois M (2018) A blood capillary plexus-derived population of progenitor cells contributes to genesis of the dermal lymphatic vasculature during embryonic development. Development 145:dev160184

Planas-Paz L, Lammert E (2014) Mechanosensing in developing lymphatic vessels. Adv Anat Embryol Cell Biol 214:23–40

Planas-PAZ L, Strilić B, Goedecke A, Breier G, Fässler R, Lammert E (2012) Mechanoinduction of lymph vessel expansion. EMBO J 31:788–804

Podgrabinska S, Braun P, Velasco P, Kloos B, Pepper MS, Skobe M (2002) Molecular characterization of lymphatic endothelial cells. Proc Natl Acad Sci U S A 99:16069–16074

Poh YC, Chen J, Hong Y, Yi H, Zhang S, Chen J, Wu DC, Wang L, Jia Q, Singh R, Yao W, Tan Y, Tajik A, Tanaka TS, Wang N (2014) Generation of organized germ layers from a single mouse embryonic stem cell. Nat Commun 5:4000

Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U (2004) Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development 131:1619–1628

Potente M, Mäkinen T (2017) Vascular heterogeneity and specialization in development and disease. Nat Rev Mol Cell Biol 18:477–494

Ramaiah KD, Ottesen EA (2014) Progress and impact of 13 years of the global programme to eliminate lymphatic filariasis on reducing the burden of filarial disease. PLoS Negl Trop Dis 8: e3319

Rehal S, Von Der Weid P-Y (2017) TNÎâARE mice display abnormal lymphatics and develop tertiary lymphoid organs in the mesentery. Am J Pathol 187(4):798–807

Rehal S, Stephens M, Roizes S, Liao S, Von Der Weid P-Y (2017) Acute small intestinal inflammation results in persistent lymphatic alterations. Am J Physiol Gastroin Liver Physiol 314:G408–G417

Sabine A, Agalarov Y, Maby-EL Hajjami H, Jaquet M, Hägerling R, Pollmann C, Bebber D, Pfenniger A, Miura N, Dormond O, Calmes JM, Adams RH, Mäkinen T, Kiefer F, Kwak BR, Petrova TV (2012) Mechanotransduction, PROX1, and FOXC2 cooperate to control connexin37 and calcineurin during lymphatic-valve formation. Dev Cell 22:430–445

Sage H, Iruela-Arispe ML (1990) Type VIII collagen in murine development. Association with capillary formation in vitro. Ann N Y Acad Sci 580:17–31

Saito N, Hamada J, Furukawa H, Tsutsumida A, Oyama A, Funayama E, Saito A, Tsuji T, Tada M, Moriuchi T, Yamamoto Y (2009) Laminin-421 produced by lymphatic endothelial cells induces chemotaxis for human melanoma cells. Pigment Cell Melanoma Res 22:601–610

Samama B, Boehm N (2005) Reelin immunoreactivity in lymphatics and liver during development and adult life. Anat Rec A Discov Mol Cell Evol Biol 285:595–599

Savetsky IL, Ghanta S, Gardenier JC, Torrisi JS, García Nores GD, Hespe GE, Nitti MD, Kataru RP, Mehrara BJ (2015) Th2 cytokines inhibit lymphangiogenesis. PLoS One 10:e0126908

Scaldaferri F, Vetrano S, Sans M, Arena V, Straface G, Stigliano E, Repici A, Sturm A, Malesci A, Panes J, Yla-Herttuala S, Fiocchi C, Danese S (2009) VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology 136:585–95.e5

Schaverien MV, Munnoch DA, Brorson H (2018) Liposuction treatment of lymphedema. Semin Plast Surg 32:42–47

Schwager S, Detmar M (2019) Inflammation and lymphatic function. Front Immunol 10:308

Shen EM, Mccloskey KE (2017) Development of mural cells: from in vivo understanding to in vitro recapitulation. Stem Cells Dev 26:1020–1041

Shi X, Richard J, Zirbes KM, Gong W, Lin G, Kyba M, Thomson JA, Koyano-Nakagawa N, Garry DJ (2014) Cooperative interaction of Etv2 and Gata2 regulates the development of endothelial and hematopoietic lineages. Dev Biol 389:208–218

Shimshoni E, Adir I, Afik R, Solomonov I, Shenoy A, Adler M, Puricelli L, Sabino F, Savickas S, Mouhadeb O, Gluck N, Fishman S, Werner L, Salame TM, Shouval DS, Varol C, Auf Dem Keller U, Podestà A, Geiger T, Milani P, Alon U, Sagi I (2021) Distinct extracellular-matrix remodeling events precede symptoms of inflammation. Matrix Biol 96:47–68

Shin M, Male I, Beane TJ, Villefranc JA, Kok FO, Zhu LJ, Lawson ND (2016) Vegfc acts through ERK to induce sprouting and differentiation of trunk lymphatic progenitors. Development 143: 3785–3795

Sivaraj KK, Dharmalingam B, Mohanakrishnan V, Jeong HW, Kato K, Schröder S, Adams S, Koh GY, Adams RH (2020) YAP1 and TAZ negatively control bone angiogenesis by limiting hypoxia-inducible factor signaling in endothelial cells. elife 9:e50770

Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, Riccardi L, Alitalo K, Claffey K, Detmar M (2001) Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med 7:192–198

Sleigh BC, Manna B (2021) Lymphedema. In: StatPearls. StatPearls, Treasure Island, FL. Copyright © 2021, StatPearls Publishing LLC

Smeltzer DM, Stickler GB, Schirger A (1985) Primary lymphedema in children and adolescents: a follow-up study and review. Pediatrics 76:206–218

Song E, Mao T, Dong H, Boisserand LSB, Antila S, Bosenberg M, Alitalo K, Thomas J-L, Iwasaki A (2020) VEGF-C-driven lymphatic drainage enables immunosurveillance of brain tumours. Nature 577:689–694

Sounni NE, Paye A, Host L, Noël A (2011) MT-MMPS as regulators of vessel stability associated with angiogenesis. Front Pharmacol 2:111

Spencer DM, Veldman GM, Banerjee S, Willis J, Levine AD (2002) Distinct inflammatory mechanisms mediate early versus late colitis in mice. Gastroenterology 122:94–105

Srinivasan RS, Dillard ME, Lagutin OV, Lin FJ, Tsai S, Tsai MJ, Samokhvalov IM, Oliver G (2007) Lineage tracing demonstrates the venous origin of the mammalian lymphatic vasculature. Genes Dev 21:2422–2432

Srinivasan RS, Geng X, Yang Y, Wang Y, Mukatira S, Studer M, Porto MP, Lagutin O, Oliver G (2010) The nuclear hormone receptor Coup-TFII is required for the initiation and early maintenance of Prox1 expression in lymphatic endothelial cells. Genes Dev 24:696–707

Stanczuk L, Martinez-Corral I, Ulvmar MH, Zhang Y, Laviña B, Fruttiger M, Adams RH, Saur D, Betsholtz C, Ortega S, Alitalo K, Graupera M, Mäkinen T (2015) cKit lineage Hemogenic endothelium-derived cells contribute to mesenteric lymphatic vessels. Cell Rep 10:1708–1721

Steiner E, Enzmann GU, Lyck R, Lin S, Rüegg MA, Kröger S, Engelhardt B (2014) The heparan sulfate proteoglycan agrin contributes to barrier properties of mouse brain endothelial cells by stabilizing adherens junctions. Cell Tissue Res 358:465–479

Stenzel D, Lundkvist A, Sauvaget D, Busse M, Graupera M, Van Der Flier A, Wijelath ES, Murray J, Sobel M, Costell M, Takahashi S, Fässler R, Yamaguchi Y, Gutmann DH, Hynes RO, Gerhardt H (2011) Integrin-dependent and -independent functions of astrocytic fibronectin in retinal angiogenesis. Development 138:4451–4463

Stewart DC, Berrie D, Li J, LIU X, Rickerson C, Mkoji D, Iqbal A, Tan S, Doty AL, Glover SC, Simmons CS (2018) Quantitative assessment of intestinal stiffness and associations with fibrosis in human inflammatory bowel disease. PLoS One 13:e0200377

Stone OA, Stainier DYR (2019) Paraxial mesoderm is the major source of lymphatic endothelium. Dev Cell 50:247–255.e3

Stritt S, Koltowska K, Mäkinen T (2021) Homeostatic maintenance of the lymphatic vasculature. Trends Mol Med 27:955–970

Stupack DG, Cheresh DA (2004) Integrins and angiogenesis. Curr Top Dev Biol 64:207–238

Stürzl M, Kunz M, Krug SM, Naschberger E (2021) Angiocrine regulation of epithelial barrier integrity in inflammatory bowel disease. Front Med 8

Stylianopoulos T, Martin JD, Snuderl M, Mpekris F, Jain SR, Jain RK (2013) Coevolution of solid stress and interstitial fluid pressure in tumors during progression: implications for vascular collapse. Cancer Res 73:3833–3841

Sweet DT, Jiménez JM, Chang J, Hess PR, Mericko-Ishizuka P, Fu J, Xia L, Davies PF, Kahn ML (2015) Lymph flow regulates collecting lymphatic vessel maturation in vivo. J Clin Invest 125: 2995–3007

Tammela T, Alitalo K (2010) Lymphangiogenesis: molecular mechanisms and future promise. Cell 140:460–476

Thyboll J, Kortesmaa J, Cao R, Soininen R, Wang L, Iivanainen A, Sorokin L, Risling M, Cao Y, Tryggvason K (2002) Deletion of the laminin alpha4 chain leads to impaired microvessel maturation. Mol Cell Biol 22:1194–1202

Torgbenu E, Luckett T, Buhagiar MA, Chang S, Phillips JL (2020) Prevalence and incidence of cancer related lymphedema in low and middle-income countries: a systematic review and meta-analysis. BMC Cancer 20:604

Trappmann B, Baker BM, Polacheck WJ, Choi CK, Burdick JA, Chen CS (2017) Matrix degradability controls multicellularity of 3D cell migration. Nat Commun 8:371

Trédan O, Galmarini CM, Patel K, Tannock IF (2007) Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst 99:1441–1454

Vaeyens MM, Jorge-Peñas A, Barrasa-Fano J, Shapeti A, Roeffaers M, Van Oosterwyck H (2020) Actomyosin-dependent invasion of endothelial sprouts in collagen. Cytoskeleton (Hoboken) 77: 261–276

Van Impel A, Zhao Z, Hermkens DMA, Roukens MG, Fischer JC, Peterson-Maduro J, Duckers H, Ober EA, Ingham PW, Schulte-Merker S (2014) Divergence of zebrafish and mouse lymphatic cell fate specification pathways. Development 141:1228–1238

Van Obberghen-Schilling E, Tucker RP, Saupe F, Gasser I, Cseh B, Orend G (2011) Fibronectin and tenascin-C: accomplices in vascular morphogenesis during development and tumor growth. Int J Dev Biol 55:511–525

Wang XL, Zhao J, Qin L, Qiao M (2016) Promoting inflammatory lymphangiogenesis by vascular endothelial growth factor-C (VEGF-C) aggravated intestinal inflammation in mice with experimental acute colitis. Braz J Med Biol Res 49:e4738

Wang S, Nie D, Rubin JP, Kokai L (2017a) Lymphatic endothelial cells under mechanical stress: altered expression of inflammatory cytokines and fibrosis. Lymphat Res Biol 15:130–135

Wang Y, Jin Y, Mäe MA, Zhang Y, Ortsäter H, Betsholtz C, Mäkinen T, Jakobsson L (2017b) Smooth muscle cell recruitment to lymphatic vessels requires PDGFB and impacts vessel size but not identity. Development 144:3590–3601

Wang G, Muhl L, Padberg Y, Dupont L, Peterson-Maduro J, Stehling M, Le Noble F, Colige A, Betsholtz C, Schulte-Merker S, Van Impel A (2020) Specific fibroblast subpopulations and neuronal structures provide local sources of Vegfc-processing components during zebrafish lymphangiogenesis. Nat Commun 11:2724

Warren AG, Brorson H, Borud LJ, Slavin SA (2007) Lymphedema: a comprehensive review. Ann Plast Surg 59:464–472

Wei B, Zhou X, Liang C, Zheng X, Lei P, Fang J, Han X, Wang L, Qi C, Wei H (2017) Human colorectal cancer progression correlates with LOX-induced ECM stiffening. Int J Biol Sci 13: 1450–1457

Wei M, Ma Y, Shen L, Xu Y, Liu L, Bu X, Guo Z, Qin H, Li Z, Wang Z, Wu K, Yao L, Li J, Zhang J (2020) NDRG2 regulates adherens junction integrity to restrict colitis and tumourigenesis. EBioMedicine 61:103068

Weiler MJ, Cribb MT, Nepiyushchikh Z, Nelson TS, Dixon JB (2019) A novel mouse tail lymphedema model for observing lymphatic pump failure during lymphedema development. Sci Rep 9:10405

Wessel F, Winderlich M, Holm M, Frye M, Rivera-Galdos R, Vockel M, Linnepe R, Ipe U, Stadtmann A, Zarbock A, Nottebaum AF, Vestweber D (2014) Leukocyte extravasation and vascular permeability are each controlled in vivo by different tyrosine residues of VE-cadherin. Nat Immunol 15:223–230

Wigle JT, Oliver G (1999) Prox1 function is required for the development of the murine lymphatic system. Cell 98:769–778

Wirtz S, Neufert C, Weigmann B, Neurath MF (2007) Chemically induced mouse models of intestinal inflammation. Nat Protoc 2:541–546

Wong HLX, Jin G, Cao R, Zhang S, Cao Y, Zhou Z (2016) MT1-MMP sheds LYVE-1 on lymphatic endothelial cells and suppresses VEGF-C production to inhibit lymphangiogenesis. Nat Commun 7:10824

Wong L, Kumar A, Gabela-Zuniga B, Chua J, Singh G, Happe CL, Engler AJ, Fan Y, Mccloskey KE (2019) Substrate stiffness directs diverging vascular fates. Acta Biomater 96:321–329

Wu X, Zhuo S, Chen J, Liu N (2011) Real-time in vivo imaging collagen in lymphedematous skin using multiphoton microscopy. Scanning 33:463–467

Wynn TA (2008) Cellular and molecular mechanisms of fibrosis. J Pathol 214:199–210

Xu Y, Yu Q (2001) Angiopoietin-1, unlike angiopoietin-2, is incorporated into the extracellular matrix via its linker peptide region. J Biol Chem 276:34990–34998

Xu Y, Liu YJ, Yu Q (2004) Angiopoietin-3 is tethered on the cell surface via heparan sulfate proteoglycans. J Biol Chem 279:41179–41188

Xue C, Zhang T, Xie X, Zhang Q, Zhang S, Zhu B, Lin Y, Cai X (2017) Substrate stiffness regulates arterial-venous differentiation of endothelial progenitor cells via the Ras/Mek pathway. Biochim Biophys Acta, Mol Cell Res 1864:1799–1808

Yang Y, García-Verdugo JM, Soriano-Navarro M, Srinivasan RS, Scallan JP, Singh MK, Epstein JA, Oliver G (2012) Lymphatic endothelial progenitors bud from the cardinal vein and intersomitic vessels in mammalian embryos. Blood 120:2340–2348

Yaniv K, Isogai S, Castranova D, Dye L, Hitomi J, Weinstein BM (2006) Live imaging of lymphatic development in the zebrafish. Nat Med 12:711–716

Yao L-C, Baluk P, Feng J, Mcdonald DM (2010) Steroid-resistant lymphatic remodeling in chronically inflamed mouse airways. Am J Pathol 176:1525–1541

Yao LC, Baluk P, Srinivasan RS, Oliver G, Mcdonald DM (2012) Plasticity of button-like junctions in the endothelium of airway lymphatics in development and inflammation. Am J Pathol 180: 2561–2575

Yuan Y, Arcucci V, Levy SM, Achen MG (2019) Modulation of immunity by lymphatic dysfunction in lymphedema. Front Immunol 10:76

Zhang X, Groopman JE, Wang JF (2005) Extracellular matrix regulates endothelial functions through interaction of VEGFR-3 and integrin alpha5beta1. J Cell Physiol 202:205–214

Zhang F, Zarkada G, Yi S, Eichmann A (2020) Lymphatic endothelial cell junctions: molecular regulation in physiology and diseases. Front Physiol 11:509

Zhen G, Cao X (2014) Targeting TGFβ signaling in subchondral bone and articular cartilage homeostasis. Trends Pharmacol Sci 35:227–236

Zhong A, Mirzaei Z, Simmons CA (2018) The roles of matrix stiffness and ß-catenin signaling in endothelial-to-mesenchymal transition of aortic valve endothelial cells. Cardiovasc Eng Technol 9:158–167

Zhou X, Rowe RG, Hiraoka N, George JP, Wirtz D, Mosher DF, Virtanen I, Chernousov MA, Weiss SJ (2008) Fibronectin fibrillogenesis regulates three-dimensional neovessel formation. Genes Dev 22:1231–1243

Zhou Q, Wood R, Schwarz EM, Wang YJ, Xing L (2010) Near-infrared lymphatic imaging demonstrates the dynamics of lymph flow and lymphangiogenesis during the acute versus chronic phases of arthritis in mice. Arthritis Rheum 62:1881–1889

Zoeller JJ, Mcquillan A, Whitelock J, Ho SY, Iozzo RV (2008) A central function for perlecan in skeletal muscle and cardiovascular development. J Cell Biol 181:381–394