BMB
Reports

BMB Rep. 2021; 54(6): 285-294
www.bmbreports.org

Invited Mini Review

A novel role of Hippo-Yap/TAZ signaling pathway in lymphatic vascular development

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The lymphatic vasculature plays important role in regulating fluid homeostasis, intestinal lipid absorption, and immune surveillance in humans. Malfunction of lymphatic vasculature leads to several human diseases. Understanding the fundamental mechanism in lymphatic vascular development not only expand our knowledge, but also provide a new therapeutic insight. Recently, Hippo-YAP/TAZ signaling pathway, a key mechanism of organ size and tissue homeostasis, has emerged as a critical player that regulate lymphatic specification, sprouting, and maturation. In this review, we discuss the mechanistic regulation and pathophysiological significant of Hippo pathway in lymphatic vascular development. [BMB Reports 2021; 54(6): 285-294]

INTRODUCTION

In mammals, there exist complementary vascular networks. Blood vasculature delivers nutrients and oxygen to cells, and the lymphatic vasculature maintains fluid homeostasis by collecting and returning interstitial fluid into the bloodstream. Additionally, lymphatic vasculature regulates lipid absorption and immune response (1, 2). Dysfunction of lymphatic vessels is associated with several human diseases such as lymphedema, Alzheimer's disease, cancer metastasis, obesity, atherosclerosis, and inflammatory diseases (3-10). A major part of the lymphatic vascular network is established during embryonic stages (2, 11). Three stepwise events regulate the lymphatic vasculature development: specification of lymphatic endothelial cell (LECs) progenitors, differentiation of LECs and formation of lymph sacs, and patterning and maturation of the lymphatic vessels. Different signaling pathways such as VEGFC-VEGFR3, NOTCH, BMP, WNT, PCP, G-protein-coupled receptors (GPCRs), ECM-integrin, and mechanotransduction signaling pathway regulate LEC identity, morphology, and behaviors via their downstream kinases, adaptor molecules, and transcriptional factors (1, 12-18).

The Hippo signaling is an evolutionarily conserved organ size-control mechanism and plays pivotal roles in maintaining tissue homeostasis by regulating cell proliferation, growth, and survival (19-22). In mammals, the central transcription factors of the Hippo pathway are Yes-associated protein (YAP) and its paralogue WW domain-containing transcription regulator (WWTR, hereinafter referred to as TAZ) (23, 24). In general, the phosphorylation status has been generally accepted as the most important regulatory mechanism for determining YAP/TAZ's subcellular localization and transcriptional activity (25, 26). The Hippo signaling can be tightly controlled by a core kinase cascade consisting of the Ste-20 family of protein kinase MST1/2, the scaffolding protein Salvador (SAV), and large tumor suppressor kinase LATS1/2 (27-32). MOB kinase activator 1A/1B (MOB1A/1B) forms a complex with LATS1/2 kinases. MST1/2 activates MOB1A/1B and LATS1/2 by phosphorylation. The tumor suppressor NF2/Merlin associates with LATS1/2 and accelerates LATS1/2 phosphorylation through the MST1/2–SAV complex. In parallel to MST1/2, MAP4K family kinases can also directly phosphorylate and activate LATS1/2 (25, 33). The wide range of intrinsic or extrinsic signals regulate the Hippo pathway that precedes to the LATS1/2-mediated YAP/TAZ phosphorylation. As a result, YAP/TAZ are located in the cytoplasm through interaction with 14-3-3, and E3 ligase β-TrCP results in the proteasome-dependent YAP/TAZ degradation (34-37). In addition, growth factor signaling or cytoskeleton rearrangement inhibits YAP/TAZ phosphorylation by suppressing the Hippo pathway and enables YAP/TAZ to translocate into the nucleus. Then, YAP/TAZ associates with TEA domain family member 1-4 (TEAD1-4) and binds with several transcription factors SMADs, RUNXs, and p63/p73 to activate the transcriptional program involved in anti-apoptosis and cell proliferation (38, 39). Recently, several studies have identified Hippo-YAP/TAZ signaling components as novel players in lymphatic vascular development by regulating LEC specification, proliferation, and migration. Therefore, in this review, we provide the current

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https://doi.org/10.5483/BMBRep.2021.54.6.020

Received 25 January 2021, Revised 23 February 2021, Accepted 3 March 2021

Keywords: Hippo signaling pathway, Lymphatic endothelial cells (LECs), Lymphatic vascular development, PROX1, TAZ, VEGF-C/VEGFR3, YAP

ISSN: 1976-670X (electronic edition)

findings concerning the Hippo-YAP/TAZ signaling pathway mediated regulation of lymphatic vessel formation and maturation.

YAP/TAZ EXPRESSION IN LYMPHATIC VASCULATURE

It has been reported that YAP/TAZ are dynamically expressed in blood vascular endothelial cells (BECs) during angiogenesis (40-42). Remarkably, TAZ expression has been reported to be higher in several types of BECs compared with YAP (42). YAP/TAZ are highly restricted in the nucleus of BECs between E10.5 to 11.5. However, YAP/TAZ are also found in the cytosol of most of the brain BECs at E14.5 (42). While YAP is mainly located in the cytoplasm in the migrating tip cells, TAZ is localized in the nuclei in the retinal blood vasculature at postnatal stage P5 (40, 41). In addition, YAP is detectable at cell-cell junctions in blood vessels of neonatal mice (43). Moreover, dynamic and differential YAP/TAZ expression patterns are observed during lymphatic vasculature development. In primary human LECs (hLECs), TAZ is expressed at a much higher level compared to YAP at the protein level. However, based on RNA-seq data, *YAP* expression is extremely high compared to *TAZ* (44). The mechanisms that regulate the post-transcriptional regulation of YAP/TAZ are still not known in LECs. TAZ is mainly located in the nuclei, but YAP is diffusely distributed in the LECs of the lymphatic plexus (45). In the newly forming lymphatic valve-endothelial cells (LV-ECs), TAZ is mainly localized in the cytoplasmic compartment at E16.5 (44, 45). However, TAZ is predominantly located in the nucleus of mature LV-EC after E17.5 (44, 46). In premature LVs CTGF and ANGPT2, the target genes of YAP/TAZ were not present, but high expression has been reported in mature LVs (44, 46). It has been proposed that YAP/TAZ have both distinct and redundant functions so they can compensate for each other in a context-dependent manner (47, 48). Single deletion of YAP or TAZ in LECs does not lead to any obvious developmental defect (44). Single deletion of TAZ leads to very mild lymphatic valve defect (45). On the contrary, genetic inactivation of both YAP/TAZ in LECs results in dramatic dilation of lymphatic vessels and structural LV deterioration (44, 45).

UPSTREAM SIGNALS REGULATING HIPPO-YAP/TAZ PATHWAY IN LECs

Over 20 years of research has firmly established that YAP/TAZ, the central players of the Hippo pathway, are the molecular determinants for organ size control (19). The multiple signaling pathways, such as mechanical stress, WNT, TGF-β, NOTCH, and VEGF, have been suggested to affect the growth-regulatory abilities of YAP/TAZ and interact with the Hippo pathway to coordinate numerous biological processes, indicating the significance of the signaling network (20). Here, we summarize the details of upstream signals that hold the potential to modulate the Hippo pathway in LECs (Fig. 1).

VEGF-C/VEGFR3 signal

While VEGF, a ligand of VEGFR2 is an essential factor for blood vessel development, VEGF-C is the major ligand that activates VEGFR3 for lymphatic vascular development (1, 49). VEGF-C/VEGFR3 interaction activates PI3K-AKT and PKC-ERK pathways to regulate LECs proliferation, survival, and migration

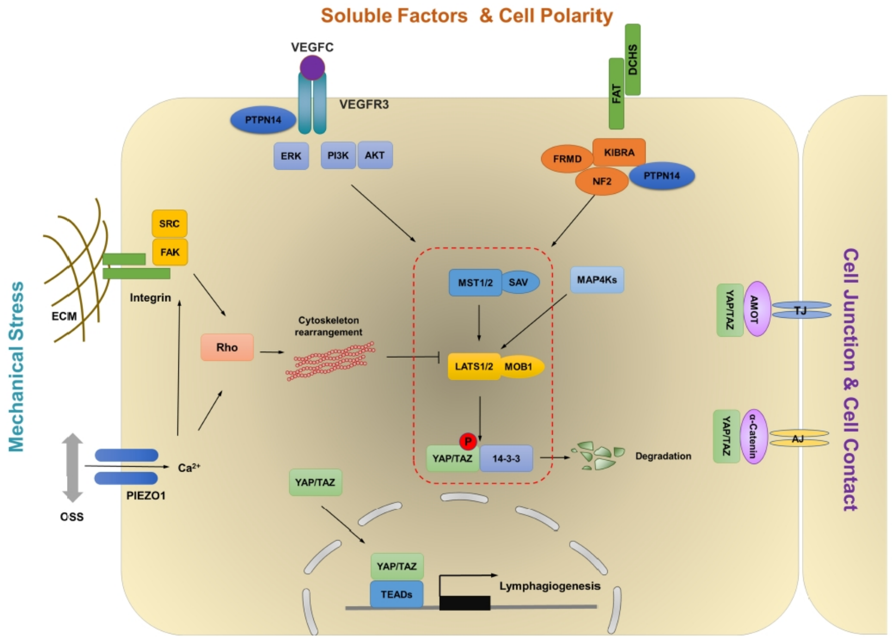

Fig. 1. Schematic diagram of the proposed model of Hippo-YAP/TAZ signaling pathway in LECs. The Hippo pathway is a kinase cascade involving MST1/2 mediated activation of LATS1/2 and LATS1/2 mediated inactivation of YAP/TAZ through phosphorylation. Multiple stimulations including VEGF-C, mechanical stress, cell density, and cell polarity may suppress upstream kinases to activate YAP/TAZ transcriptional activity.

(50). It has been reported that VEGF-C treatment increased phospho-LATS1 and facilitated cytoplasmic YAP whereas VEGFR3 knockdown promoted nuclear localization of YAP, thereby suggesting that VEGF-C activates the Hippo signaling to repress YAP/TAZ in hLECs (45). However, Hogan and colleagues suggested that Vegfc can promote nuclear Yap1 in a zebrafish model (51). In addition, VEGF-C decrease phospho-YAP and phospho-LATS1 in low confluent hLECs *in vitro* and YAP/TAZ activity is downregulated in *Vegfc*$^{+/-}$ embryos (44). The activation of VEGF/VEGFR2 signaling induces PI3K-AKT and MEK-ERK signaling pathways that lead to the inhibition of MST1/2 and LATS1/2 in (52). Although Vegfc activates Yap1 in zebrafish via ERK activation, the detailed molecular mechanisms downstream of VEGF-C/VEGFR3 in LECs are still unknown. Interestingly, KRAS/MAPK pathway which is triggered by VEGF-C/VEGFR3 can induce YAP expression during skin cancer progression (53). Overall, the current data suggest that YAP/TAZ are regulated by the VEGF-C/VEGFR3 signaling pathway in a high context- and cell type-specific manner.

Cell polarity and cell-cell contact

Elucidating the critical roles of apical–basal cell polarity in the regulation of the Hippo pathway provides insight to better understand the link between the cellular structural components and growth-regulatory mechanism. At the adherent junction, the FERM domain proteins Merlin (Mer) and Expanded (Ex) have been reported to connect the transmembrane proteins to the cytoskeleton. Mer and Ex genetically and functionally co-operate to mediate activation of LATS1/2 and consequent inhibition of YAP/TAZ (54). Moreover, the atypical cadherin Fat has emerged as an upstream regulator of Ex, which promotes its junctional localization and stability (55). However, the FAT4-DCHS1 signaling is essential for vertebral growth in YAP/TAZ independent manner (56). Mutations in FAT4 have been reported in Hennekam lymphangiectasia-lymphedema syndrome, features of which include lymphedema, lymphangiectasia, and mental retardation (57). Fat4 inactivation leads to dysmorphic lymphatic valves and impaired polarization of LECs in response to the flow. However, YAP/TAZ target genes are not affected by the loss of Fat4 (58). VANGL2 is also a core PCP component and YAP activity is reduced in the lung airways of Vangl2^{Lp} embryos (59). *Looptail* embryos, Vangl2 mutants, possess lymphatic valve maturation defect (13). It is still unclear whether the PCP pathway coordinates with the Hippo pathway in the lymphatic vasculature.

The Hippo-YAP/TAZ pathway regulates several cellular processes in response to cell-cell contact. Cell-adherent molecules are the regulator of the Hippo pathway. In high cell density, the Hippo pathway is activated and LATS1/2 kinase activity is increased, thereby leading to YAP/TAZ phosphorylation (36). YAP activity could be regulated by VE-cadherin-mediated cell-cell contact in blood endothelial cells via PI3K-AKT (43, 60). We observed that YAP/TAZ activity was down-regulated in high cell density in *in vitro* hLECs, thereby indicating conservation of contact inhibition in LECs. During LV maturation, LV-ECs have discontinuous and low-density cell-cell junctions (13) and LV-ECs have high YAP/TAZ activity (44). Deletion of VE-cadherin from LECs leads to the up-regulation of YAP/TAZ activity in the dermal and mesenteric LECs (61). However, YAP/TAZ activity is downregulated in the intestinal LECs (61, 62).

Tyrosine phosphatase PTPN14 which is associated with Choanal Atresia-Lymphedema interacts with VEGFR3 and inhibits its downstream signaling cascade (63). PTPN14 also has an interesting relationship with the Hippo signaling pathway; it interacts with the Kibra and induced LATS1 activation to negatively regulate oncogenic YAP activity (64, 65). In addition, PTPN14 protein level has been reported to be elevated in response to an increase in cell density; the protein regulates nucleus-to-cytoplasm translocation of YAP in MCF10A cells (66).

Physical signal

Accumulating evidence has suggested YAP/TAZ as the central mechanosensor and mechanotransducer in response to several kinds of mechanical stresses including shear stress, stiffness, and cell geometry. These physical signals regulate the localization and activities of YAP/TAZ to coordinate complex organ architectures (21, 67, 68). BECs are constantly exposed to mechanical forces generated by blood flow which affects cell proliferation and morphogenesis. Laminar shear stress (LSS) inactivates YAP/TAZ, whereas oscillatory shear stress (OSS) stimulates YAP/TAZ activity in BECs (69, 70). However, a following report suggested that even LSS can transiently activate YAP in BECs (71), and flow patterns could control the localization of YAP (72). Lymph flow generates shear stress in lymphatic vessels; the stress has been reported as critical for lymphatic vascular development (14, 73). LSS can enhance the proliferation and sprouting of LECs through ORAI1 mediated calcium influx and inhibition of NOTCH1 (73). LSS also enhances VEGF-C signaling through unknown mechanisms (74). Ca^{2+} entry through the ORAI channel can inhibit YAP/TAZ human glioblastoma cell lines (75). In addition, OSS is critical for lymphatic valve formation (12, 46). Shear stress sensing molecules such as PIEZO1 and VE-cadherin regulate lymphatic valve development (61, 62, 76, 77). PIEZO1 activation elicits transient Ca^{2+} influx and positively regulates nuclear localization of YAP in neural stem cells and osteoblasts (78-80). OSS increases YAP/TAZ activity and promotes nuclear localization of YAP/TAZ in in vitro cultured hLECs (46).

Physical changes induced by extracellular stiffness induce cytoskeleton rearrangement through actin remodeling, which controls the Hippo pathway in response to the activity of Integrin, Rho-GTPase, or FAK-SRC (67, 68, 81, 82). In cultured hLECs, soft matrix inhibits YAP/TAZ but promotes nuclear accumulation of GATA2 (83). In human mammary epithelial cells stretched by fluid pressure, YAP/TAZ can be activated thereby resulting in the entry of cells into the proliferative S phase (84). Migrating LECs are mechanically stretched by interstitial fluid pressure thereby resulting in the swelling of the interstitium (85). Integrin β1, a key component of ECM stiffness dependent YAP/TAZ activation, is necessary for inducing response to mechanical stretch to enhance VEGF-C/VEGFR3 signaling during LECs migration (85).

G-protein-coupled receptors (GPCRs) signaling

Yu and colleagues have accomplished a conceptual development in the regulation of the Hippo-YAP/TAZ pathway and G-protein-coupled receptors (GPCRs), the largest group of membrane receptors. GPCRs function as a critical upstream regulator in the Hippo pathway and relay the extracellular signal to Hippo signaling components (86). Depending on the type of ligands, GPCRs activate different types of heterotrimeric G-protein, thereby causing differential regulation of the Hippo signaling. Lysophosphatidic acid (LPA) or sphingosine 1-phosphate (S1P) promotes YAP/TAZ activation through G12/13-dependent LATS1/2 inhibition while epinephrine or glucagon suppresses YAP/TAZ activation by Gs signaling (86). Adrenomedullin (AM) and its receptor complex, the G protein-coupled receptor CLR (calcitonin receptor-like receptor; Calcrl) and Ramp2, play critical roles in lymphatic development during embryogenesis and maintenance of normal lymphatic function in adults (15, 87). LPA, a positive regulator of YAP/TAZ activity, is essential for lymphatic vascular development (88, 89). PROX1 and LYVE1 expression are induced by LPA stimulation in BECs (90). Also, S1P promotes lymphangiogenesis by activating S1P receptor 1 (S1PR1) which couples stringently to the Gi protein (91) and S1PR1 signaling is active in mature and quiescent lymphatic vessels during development (74). Taken together, several studies suggest that GPCRs signaling pathway regulates LEC proliferation and migration, and determines lymphatic vessel integrity and permeability. Therefore, it will be worthy to explore the potential cross-talk between GPCR and Hippo-YAP/TAZ signaling pathway.

WNT signaling

The WNT/β-catenin signaling is a critical regulator that is involved in embryo development and tissue homeostasis. Abnormal regulation of WNT signaling causes diverse human diseases, including cancer and neurodegenerative disorders (92, 93). In the canonical WNT pathway, β-catenin is a major transcription factor activating WNT-responsive target gene expression. Without WNT stimulation, β-catenin is sequestered and phosphorylated by destruction complex containing Axin, APC, and GSK3β, followed by β-TrCP-mediated proteasomal degradation in the cytosol. In response to the WNT stimulus, accumulated β-catenin translocates into the nucleus and ultimately activates WNT transcriptional program (94). Interestingly, growing evidence has suggested that WNT and Hippo pathways integrate and converge in the multiple layers of signaling pathways to respond to physiological inputs or alterations (95). Indeed, TAZ is known to functionally mediate WNT signaling (96). Also, YAP/TAZ can be seized by β-catenin destruction complex via physical interaction with Axin (97). In addition to the canonical WNT pathway, noncanonical WNT ligands regulate YAP/TAZ activation via $G\alpha_{12/13}$-Rho-LATS signaling (98). Both canonical and non-canonical WNT signaling are critical for lymphatic vascular development (12, 99, 100). It will be interesting to elucidate whether cross-talk between WNT-YAP/TAZ signaling is involved in this process.

NOTCH signaling

NOTCH signaling plays a central role in various biological processes and is activated by direct cell-cell communication between the NOTCH receptors and their ligands including Jagged and Delta-like. After the binding, the NOTCH receptor can be cleaved sequentially and converted into a NOTCH intracellular domain (NICD) that acts as a transcription factor to activate the NOTCH-responsive target gene (101, 102). YAP regulates expression of NOTCH receptors and their ligand Jagged1. In turn, NICD augments YAP/TAZ protein stability and creates a positive loop for tumor development (103, 104). NOTCH inhibits lymphatic development by repressing PROX1 expression (105). Genetic inactivation of Notch1 in LECs of mouse embryos leads to enlarge lymph sac and increase LEC populations (16, 106). However, inactivating the DLL4/NOTCH signaling using blocking antibodies leads to decline of lymphatic vessel density (107). Likewise, *Dll4*$^{+/-}$ mice have reduced lymphatic vascular density (74). The role of NOTCH in LECs is still not fully resolved. The molecular mechanism by which NOTCH pathway controls PROX1 is a key step that remains to be identified. Certainly, further work is necessitated to determine whether the NOTCH-NICD-YAP/TAZ positive feedback loop operates in the lymphatic vasculature.

HIPPO-YAP/TAZ PATHWAY IN LYMPHATIC VASCULAR DEVELOPMENT

Role of Hippo-YAP/TAZ signaling in the specification of lymphatic endothelial cell progenitors

Most of the embryonic LEC progenitors derive from the cardinal veins (108-110). In mouse embryos, around E9.5, a unique group of venous endothelial cells starts to express PROX1 and becomes LEC progenitors. The homeobox transcription factor PROX1 not only controls LEC cell-fate determination but also maintains their identity (49, 109, 111, 112). It has been known that COUP-TFII (111) and SOX18 (113) are required to activate PROX1 expression by binding directly to the PROX1 promoter. On the other hand, NOTCH signaling inhibits PROX1 expression during LEC cell-fate specification (16, 106). A Positive feedback loop between PROX1-VEGFR3 is necessary for controlling the LEC specification and for preserving LEC identity (114, 115) (Fig. 2). Koh and colleagues for the first time reported the presence of YAP/TAZ in the cytoplasm of most of the LEC progenitors (45). Activation of YAP/TAZ in cultured hLECs leads to down-regulation of PROX1 while knocking down of YAP/TAZ increases PROX1 expression (45). Hyperactivation of YAP/TAZ using *Prox1*-CreERT2;*Lats1*$^{f/f}$;*Lats2*$^{f/f}$ mouse demonstrated a reduction in the number of Prox1$^+$ LECs in cardinal vein thereby

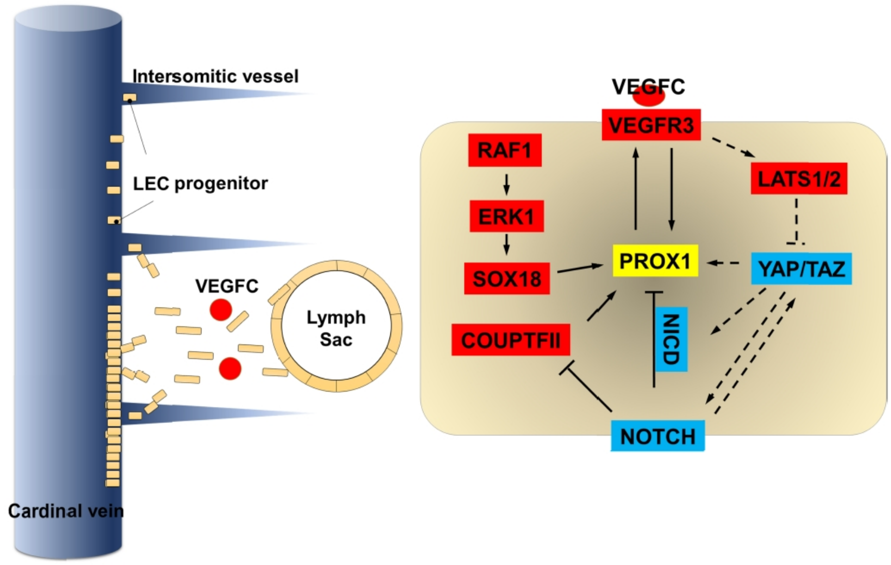

Fig. 2. Schematic representation of the molecular mechanisms controlling lymphatic specification and sprouting. PROX1 is a key transcription factor that drives lymphatic cell fate specification. Lymphatic endothelial progenitor cells expressing PROX1 arise from the cardinal veins (CV) and intersomitic veins. After specification, LECs migrate out from the CV in a VEGF-C/VEGFR3 dependent manner and form lymph sac. Hippo-YAP/TAZ signaling regulates PROX1 expression during LEC specification and sprouting.

indicating that Hippo signaling activates PROX1 expression and promotes LECs specification (45). However, Yap1 localization has been reported to be dynamically altered in parachordal LECs in zebrafish (51). Interestingly, yap1 mutants show the normal specification of lymphatic progenitors and yap1 is not necessary for specification in the zebrafish model (51).

Role of Hippo-YAP/TAZ signaling in lymphatic endothelial cell migration

In mouse embryos, around E10.5, LEC progenitors start to migrate out from the cardinal vein into mesenchyme as loosely connected spindle-shaped LECs. These LECs form lumenized lymphatic structure such as the lymph sac, the peripheral longitudinal lymphatic vessel, and the primordial thoracic duct around E11.5 (116, 117). Sprouting process of LECs is governed by VEGF-C/VEGFR3 in both mice and zebrafish (118-120) (Fig. 2). It appears that VEGF-C/VEGFR3 signaling represses YAP/TAZ activity via LATS1 phosphorylation *in vitro* (45). Hyperactivation of YAP/TAZ at E10.5 reduced Prox1 expression thereby leading to a decrease in lymph sac size (45). On the other hand, *yap1* mutant showed abnormal cellular sprouting in zebrafish (51). Hogan and colleagues suggested that Vegfc promotes nuclear Yap1 with subsequent regulation of LECs proliferation (51). During migration, LECs are exposed to a soft ECM environment, thereby leading to a decrease in YAP/TAZ activity and activation of GATA2-dependent VEGFR3 expression (83). However, migrating LECs are mechanically stretched because of high interstitial fluid pressure (85). Integrin β1, a key component of ECM stiffness dependent YAP/TAZ activation, is necessary for responding to mechanical stretch to enhance VEGF-C/VEGFR-3 signaling during LECs migration (85).

Role of Hippo-YAP/TAZ signaling in dermal lymphatic vascular development

The first lymphatic vessels reach the skin from the jugular lymph sac around E12.5. Then, arising superficial lymphatic vessels on the lateral side of the embryo actively move towards until they reach the dorsal midline around E15.5-E16.5. (108, 121, 122). They show honeycomb-like structure in the plexus region and have actively sprouting tips in the migratory front region, reflecting a dynamic process in lymphatic vascular patterning. The molecular mechanisms regulating formation of the dermal lymphatic vasculature remain incompletely understood. However, PROX1, VEGFC, FOXC2, GATA2, and NRP2 are recognized to be necessary for the dermal lymphatic development (3, 118, 123-125). Koh and colleagues demonstrated that around E16.5, YAP/TAZ are very less expressed in the tip LECs but TAZ are nucleo-cytoplasmically located in LV-ECs in the plexus (45). Conditional deletion of YAP/TAZ in LECs from E11.5 causes enlarged, ballooned, and mispatterned lymphatic vessels with no lymphatic valves. Genetical inactivation of LATS1/2 blocks lymphatic sprouting and leads to the formation of dysmorphic lymphatic vessels (45). *Lyve1-Cre*;*Yap*$^{f/f}$;*Taz*$^{f/f}$ embryos have defective lymphatic vasculature with dilated vessels, fewer branch points, and migration defect around E18.5 (44). Therefore, Hippo-YAP/TAZ signaling pathway is unquestionably essential for dermal lymphatic vascular development.

Role of Hippo-YAP/TAZ signaling in lymphovenous and lymphatic valve development

Lymph returns to the blood circulatory system particularly through four lymphovenous valves (LVVs) (126, 127). They start forming around E12 at the junction of the jugular and subclavian veins (128, 129). The development of LVV starts

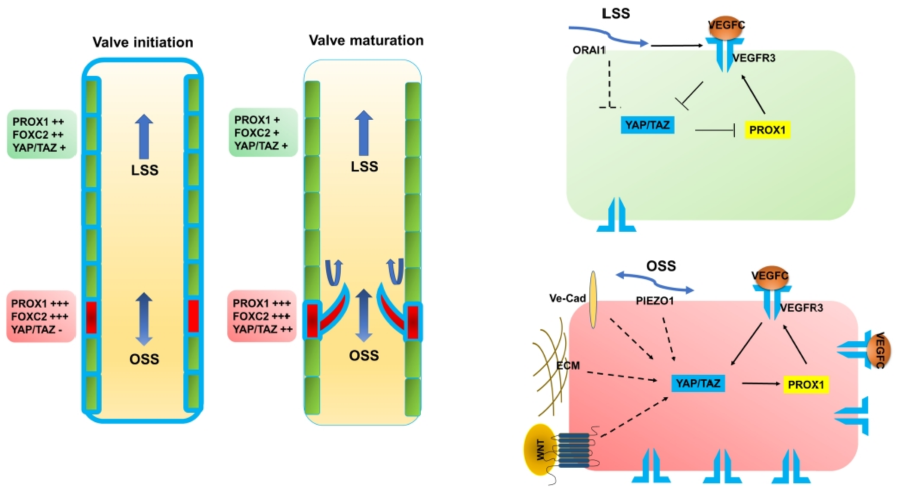

Fig. 3. A proposed mechanism of Hippo-YAP/TAZ signaling pathway function in lymphatic valve (LV) formation. LVs develop in a step-wise manner. LV-ECs are specified in red and LECs are in green. Blue lines indicate VEGFR3. Briefly, during the initiation stage, OSS promotes high PROX1 and FOXC2 expression in LV-ECs. During the valve maturation stage, LV-ECs orient perpendicular to the flow and migrate into the vessel lumen. Then, LV-ECs elongate to form a bi-layered leaflet with a thick extra-cellular matrix (ECM). YAP/TAZ activities are gradually increased during the maturation stage, thereby indicating that Hippo YAP/TAZ signaling pathway is regulated by a multitude of signaling mechanisms for LV development.

with the formation of two distinct cell populations. LECs from the lymph sacs and LVV-forming endothelial cells (LVV-ECs) from the veins interact to build the LVVs. LVV-ECs quickly aggregate again and invaginate into the vein to create valve leaflets around E12.5. Then, LVVs experience gradual maturation by assembling mural cells to the gap between the LVV-ECs between E14.5 to E16.5. The expression of PROX1, GATA2, and FOXC2 are increased in LVV-ECs and strong expression of VEGFR3 is remained in the LECs that create LVVs as well (128). YAP/TAZ and CTGF are almost absent in LVV-ECs between E12.0 to E14.5 but enriched around E16.5 (44). Consistent with the expression data, *Lyve1-Cre*;*Yap*$^{f/f}$;*Taz*$^{f/f}$ embryos lack any obvious morphologic defects at E14.5 in LVVs. However, mutants with the complete absence of LVVs at E17.5 indicate that YAP/TAZ activity progressively augments during LVV maturation and YAP/TAZ are necessary for preserving LVV-ECs. We also found that YAP/TAZ positively control PROX1 expression in LVV-ECs (44).

Skin and mesentery lymphatic valves (LVs) start developing around E15.5-E16.5 (18, 130). Differentiation of PROX1^{high}, FOXC2^{high}, and GATA2^{high} LV-ECs is the opening stage of LV development (Fig. 3). OSS generated by lymph flow is one of the most critical factors for FOXC2 and GATA2 expression (14, 131) along with activation of NFATc1 (14) and Wnt/β-catenin signaling (12, 100). The highly elongated LV-ECs line up along the wall of lymphatic vessels at E16.5, and then they aligned perpendicular to lymph flow at E17.5. ECM molecules such as collagen IV, laminin-α5, fibronectin (FN)-EIIIA, and EMILIN1 are accumulated in between the LV-EC layers around E17.5 (14, 18, 130, 132). Next, the LV-EC layers stretch along the direction of the lymph flow to produce mature LV leaflets after E18.5 (129, 133). At E16.5, TAZ is mainly localized in the cytoplasm (45) in LV-Ecs; however, at the maturation stage, TAZ appears to be located in the nucleus (44, 46). E18.5 of *Lyve1-Cre*;*Yap*$^{f/f}$;*Taz*$^{f/f}$ embryos show dilated lymphatic vessel with no LVs and immature phenotype with strong expression of LYVE1, VEGFR3, and PROX1 (44). While deletion of YAP/TAZ after birth using tamoxifen delivery system from P1 to P7 leads to a reduction in the LV number with high expression of FOXC2 and PROX1, hyperactivation of YAP/TAZ causes a decrease in LV number with low expression of PROX1 and Integrin-α9 (45). Therefore, a balance in YAP/TAZ activity is important for maintaining the lymphatic valve.

CONCLUDING REMARKS

In summary, although our understanding of lymphatic vessel functions under physiological or pathological conditions has improved in the past decade, many questions remain unclear. Therefore, it has been considered that the identification of lymphangiogenic modulators and a clear understanding of the involved signaling pathways will provide opportunities to develop therapeutic targets for lymphatic diseases. Among the factors, PROX1 and VEGF-C/VEGFR3 signaling are the most critical regulators of lymphatic vascular development. The Hippo-YAP/TAZ signaling pathway has been established as a key mechanism of regulation of organ size and tissue homeostasis. Recent studies reveal that YAP/TAZ are the vital molecules of the PROX1/VEGFR3 feedback loop in LEC specification, migration, and LV maturation. Based on the current Hippo signaling pathway, it has been hypothesized that MAP4K family kinases acting in parallel to the MST1/2-SAV1 complex can phosphorylate LATS1/2 to inactivate YAP/TAZ in a context-dependent manner. We generated SAV1 conditional knockout mice using two different Cre lines (*Lyve1-Cre*;*Sav1*$^{f/f}$ and Tie2-Cre;*Sav1*$^{f/f}$); the mutants showed no obvious phenotypes in the lymphatic vasculature. Interestingly, Czech and colleagues showed that mice constitutively lacking Map4k4 displayed LVs defect and lymphatic flow disorder with increased Prox1 expression (134). Besides VEGF-C and mechanical stress such as EMC stiffness and shear force, multiple signaling pathways are able to control the development of lymphatic vasculature. It must be elucidated whether YAP/TAZ could be regulated by these signaling pathways in a functionally related manner. Moreover, Hippo pathway molecules have been considered as therapeutic targets in several human diseases. Therefore, it is hypothesized that targeting those molecules will provide new therapeutic strategies to cure lymphatic disease in the future.

ACKNOWLEDGEMENTS

We sincerely apologize as we are unable to cite multiple key research papers due to space limitations. We thank Dr. R. Sathish Srinivasan for his insightful comments. This work is supported by the grant from the National Research Foundation of Korea (2020R1F1A1060680) to B. Cha (2020R1C1C100705011), S. Moon, and (2021R1A2C4001704) W. Kim.

CONFLICTS OF INTEREST

The authors have no conflicting interests.

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