Endothelial-epithelial communication in polycystic kidney disease: role of vascular endothelial growth factor signalling

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Abstract

Whereas targeting the cyst epithelium and its molecular machinery has been the prevailing clinical strategy for polycystic kidney disease, the endothelium, including blood vasculature and lymphatics, is emerging as an important player in this disorder. In this Review, we provide an overview of the structural and functional alterations to blood vasculature and lymphatic vessels in the polycystic kidney. We also discuss evidence for vascular endothelial growth factor signalling, otherwise critical for endothelial cell development and maintenance, as being a fundamental molecular pathway in polycystic kidney disease and a potential therapeutic target for modulating cyst expansion.

Introduction

Autosomal dominant (AD) polycystic kidney disease (PKD) affects around 1 in every 1000 individuals [1]. In ADPKD, mutations in *PKD1* and *PKD2*, encoding for polycystin 1 and polycystin 2 respectively, lead to the growth of fluid-filled cysts in the kidney [1]. This results in a loss of normal renal structure and function, often resulting in end-stage kidney disease (ESKD). Polycystin 1 and polycystin 2 localize to the cilia of renal tubular epithelial cells; facilitating mechanosensation in response to fluid flow [2]. Dysfunction of these proteins in ADPKD decreases intracellular calcium signalling, elevates intracellular cyclic adenosine monophosphate (cAMP) and activates protein kinase A-dependent gene transcription [3]. Subsequent hyperproliferation of tubular epithelium [4] and chloride-driven fluid secretion [5] drive cyst formation and expansion with loss of normal kidney structure and function. Thus, targeting the cyst epithelium and aberrant cAMP signalling has been the prevailing clinical strategy for ADPKD. This has led to the first clinically approved drug for ADPKD, tolvaptan, a vasopressin receptor 2 antagonist that modulates cAMP signalling in aberrant kidney tubules [6,7]. However, the effect of tolvaptan on slowing kidney cyst growth appears incomplete and the induction of nephrogenic diabetes insipidus is a major side effect; resulting in increased thirst and frequency in the need to pass urine [6]. Therefore, there is a need to develop further treatments for PKD, particularly those that target alternative non-epithelial mechanisms to limit cyst growth.

Insights from cancer therapeutics may provide alternative treatment strategies for PKD. Jared Grantham, to whom this special edition of *Cellular Signalling* is dedicated, drew parallels between PKD and cancer [8,9]. PKD and cancer share many common features, including proto-oncogene activation [10], modulation of the mammalian target of rapamycin cascade [11], changes to cell cycle regulators [12] and alterations in growth factor and receptor tyrosine kinase signalling [13]. Here, we explore another parallel between PKD and cancer: the blood vasculature and lymphatics. Tumour growth is a highly active process which requires oxygen and nutrients provided by the surrounding microvasculature [14]. As

cyst growth is also associated with changes in cellular metabolism [3,15], it could be postulated that remodelling of kidney blood vasculature may be required to support PKD [16]. Similarly, as remodelling of lymphatic vessels in cancer may have consequences for the dissemination of [17] and immune response to tumour cells [18], structural and functional changes to lymphatics in PKD may influence cyst expansion [19].

In this Review, we first explore how the renal microvasculature is altered in PKD, examining both blood vessels and lymphatics. We also outline how molecular signalling between endothelial and epithelial cells might contribute to cyst formation and growth. Finally, we describe recent studies which have shown how targeting the microvasculature using vascular endothelial growth factors might be a promising new therapeutic avenue for PKD.

Renal blood vasculature and lymphatics in PKD

Structural changes to the renal blood vasculature in PKD

The kidney is a highly vascularised organ and contains several structurally and molecularly distinct populations of endothelial cells, each of which are adapted to their specific and unique functions [20,21]. Of particular importance in PKD are the endothelial cells of the peritubular capillaries that surround the kidney tubules in the renal cortex. Peritubular capillaries are highly permeable to facilitate the reabsorption of glomerular filtrate into the circulation [22]. In ADPKD patients (aged between 40 and 55 years old with ESKD), angiography and corrosion casting with scanning electron microscopy have revealed that the peritubular capillaries lose their normal reticular patterning and become spiral-shaped, tortuous and dilated [23,24]. Similar findings were observed in *Pkd1*$^{nl/nl}$ mice, a mouse model of ADPKD, carrying two hypomorphic alleles of *Pkd1*. *Pkd1*$^{nl/nl}$ mice have an abnormal pericystic network of vessels present from as early as one postnatal day of age, which then becomes more disorganised as cystogenesis progresses towards renal failure; occurring at three weeks in this model [25]. Some studies have additionally quantified the numbers of renal blood vessels in animal models of PKD. Ten week old *Pck* rats, harbouring a mutation

in *Pkdh1*, encoding for fibrocystin, the gene mutated in autosomal recessive (AR)PKD, have significantly reduced blood microvascular density in both the renal cortex and medulla, accompanied by an increase in glomerular diameter as assessed by micro-computed tomography [26]. Similarly, photoacoustic imaging of eight day old *Pkd1*$^{nl/nl}$ mice revealed a reduction in the number of branch points and vessel segments compared with wild-type mice [27]. Further quantitative studies are warranted at multiple time-points of PKD models to provide a more detailed description of how the renal blood vasculature is altered as the disease progresses, but it is clear that the patterning of renal blood vasculature is abnormal around cysts in both animal models of PKD and in the human condition.

Structural changes to the renal lymphatic vasculature in PKD

The kidney also contains a network of lymphatic capillaries which have been implicated in the progression of PKD. One of the most commonly ascribed functions of lymphatics is to clear excessive tissue fluid from the interstitial compartment of organs [28]. Lymphatic capillaries have a unique structure to facilitate this role, being composed of overlapping lymphatic endothelial cells, held together by button-like junctions [29,30] and are connected to surrounding extracellular matrix by anchoring proteins [31,32]. When excessive tissue fluid builds up in the organ, the extracellular matrix expands, causing the anchoring proteins to pull adjacent lymphatic endothelial cells apart. The junctions between lymphatic endothelial cells subsequently open allowing tissue fluid, cells and small molecules to enter the lymphatic system [33]. Whereas interstitial fluid balance in the renal medulla is regulated by vasa recta, it is proposed that lymphatics and peritubular capillaries regulate this balance in the cortex and are responsible for the clearance of solute in the cortical interstitium.

A number of reports have identified structural defects of lymphatics in animal models with loss-of-function mutations in genes encoding polycystins. For example, the main lymphatic vessels in the midline of developing zebrafish fail to form in fish with a mutation in *pkd1a*, a duplicate gene encoding polycystin in zebrafish [34]. Comparatively, genetic knock-out of

Pkd1 or *Pkd2* in mice results in oedema and early lethality; ascribed to lymphatic defects due to blood-filled lymph sacs, malformation of skin lymphatics and an absence of structural heart defects [35]. Recently, we also reported structural defects to kidney lymphatics in a slow progressing model of ADPKD: the *Pkd1*$^{RC/RC}$ mouse [36], which mimics a human disease variant in *PKD1* (pR3277C). Using wholemount immunofluorescence, optical clearing and high-resolution 3D imaging [37], we identified significant reductions in the number of lymphatic vessel branches relative to kidney volume, and the percentage occupancy of kidney volume by lymphatics in *Pkd1*$^{RC/RC}$ mice, when compared with wild-type littermates at the end of murine embryonic gestation (**Figure 1**) [19]. In this model, lymphatic vessels and corticomedullary cysts were found in close proximity, indicating the possibility of fluid transport and molecular communication between lymphatic endothelial cells and cyst epithelium [19]. These studies collectively suggest that mutations in ADPKD-causative genes result in abnormalities in lymphatic formation, which may subsequently reduce the effectiveness of fluid clearance and drive the expansion of cysts in PKD.

Intrinsic changes to the blood and lymphatic vasculature in PKD

A possible explanation for the structural changes to the renal vasculature in PKD is the consequence of cysts occupying the renal parenchyma. As cysts expand, they may distort the architecture of the kidney, leading to abnormalities in the adjacent blood vasculature and lymphatics. Distortion of the blood vasculature may lead to a reduction in blood flow, increased renovascular resistance and decline in renal filtration capacity [38]. Similarly, obstruction of lymphatic vessels may reduce the clearance of interstitial fluid and pro-inflammatory immune cells and precipitate renal inflammation and fibrosis. However, this does not explain findings in rodent PKD, including the structural alterations to blood vasculature and lymphatics in the early stages of cystogenesis [19,25] or the absence of any correlation between the degree of cyst number or size and the changes in overall vessel density [26]. These collectively suggest that there may be more to the vascular phenotypes in PKD than distortion from enlarging renal cysts.

An alternative explanation for malformations of the renal vasculature in ADPKD is the direct consequences of mutations in *PKD1* or *PKD2* on the vascular endothelium. Polycystin 1 is expressed in aortic endothelial cells, small vessel endothelium and blood capillaries during mouse development [39,40], whereas polycystin 2 has been detected in murine adult femoral arteries and endothelial cells isolated from embryonic aortas [41]. Additionally, polycystin 1 and polycystin 2 are expressed in human immortalised and mouse primary lymphatic endothelial cells [35]. Both polycystin 1 and polycystin 2 mRNA and protein have been detected in skin lymphatics, with reduced expression in patients with lymphatic malformations [42]. Mounting evidence from studies knocking out endothelial cell polycystin *in vitro* and *in vivo* suggests that it has cell-autonomous requirements in the development of blood vasculature and lymphatics. Short-hairpin RNA-mediated knock-down of *PKD1* or *PKD2* from human umbilical vein endothelial cells or lymphatic endothelial cell lines impairs the formation of capillary-like structures with fewer branch points in culture [35], with *PKD1* knock-down also resulting in disorganisation of stress fibres, adherens junctions and defects in lymphatic endothelial cell polarity and migration [34]. Targeted deletion of *Pkd1* or *Pkd2* in endothelial cells using *Tie2-Cre* recombinase mice results in developmental abnormalities with a reduced number of viable mice being born [43]. This model presents with polyhydramnios and a decreased vascular density in the placenta. No overt changes were detected in the embryonic kidney or heart, but detailed vascular quantification was not performed in these organs [43]. Mouse embryos examined at embryonic day 14.5, with deletion of *Pkd1* in both blood and lymphatic cells using a *Sox18-Cre* recombinase transgenic line, displayed a normal blood vasculature but a reduction in the number and width of skin lymphatics [34]. However, the kidney was not examined in this study. Further mechanistic studies of endothelial deletion of *Pkd1 and Pkd2* in the kidney, ideally using inducible *Cre* recombinase lines to bypass any developmental abnormalities, are warranted.

Vascular endothelial growth factors in PKD

Overview of vascular endothelial growth factor signalling

The finding that blood vessels and lymphatics are malformed in PKD has raised interest in the potential role of vascular growth factors, which signal through their respective receptor tyrosine kinases enriched on endothelial cells, and are required for blood vascular and lymphatic development and function [44]. An attractive hypothesis is that modulation of vascular growth factor signalling may provide a novel non-epithelial therapeutic direction for PKD, through remodelling the pericystic blood vasculature and lymphatics and exerting paracrine, cellular or metabolic consequences on cyst growth [16]. Most studied of the vascular growth factors is the vascular endothelial growth factor (VEGF) family (**Figure 2**), composed of VEGF-A, VEGF-B, VEGF-C, VEGF-D, the virally encoded VEGF-E and placental growth factor (PIGF) [45]. VEGF-A is the main regulator of angiogenesis; the process of new blood vessel formation from pre-existing vessels. Multiple isoforms of VEGF-A exist, generated by alternative splicing, which differ in their ability to bind heparin [46]. The main pro-angiogenic isoform VEGF-A_{165} (VEGF-A_{164} in mice) binds and phosphorylates the receptor VEGFR-2, promoting the survival, migration, proliferation, permeability and dilation of blood vessels [45]. Binding of VEGF-A to VEGFR-1 and soluble fms-like tyrosine kinase 1 fine-tunes angiogenic signalling, both during development and in pathological contexts [47]. Conversely, the growth of lymphatic endothelial cells is regulated by VEGF-C and VEGF-D through their binding and activation of VEGFR-3 [48]. VEGF-C promotes the proliferation and expansion of lymphatic vessels, termed lymphangiogenesis, during both development and disease [49], whereas VEGF-D is considered to have a more important role in inflammation-induced lymphangiogenesis [50]. VEGF-C can also bind with a lower affinity to VEGFR-2 and subsequently modulate angiogenic responses in blood vessels [51].

VEGF-A signalling in polycystic kidneys

VEGF-A has been identified as a critical molecular player in PKD. In healthy adult kidneys, VEGF-A is expressed in the proximal and distal cortical tubules and most abundantly in the loop of Henle’s thick ascending limb [52,53]. VEGF-A is critical for maintaining the health of

the surrounding peritubular capillaries, which express VEGFR-2 [52]. In adult mice specifically lacking tubular *Vegfa*, there was a reduction in peritubular capillary density and polycythaemia [52], suggesting that crosstalk between tubular epithelium and endothelial cells, mediated by signalling between VEGF-A and VEGFR-2, is critical for peritubular capillary health and maintenance. VEGF-A protein has been immunolocalised to the cyst epithelial cells in tissue sections from kidneys obtained from either ADPKD patients with ESKD [54] or 12-week-old male *Cy/+* rats; which are heterozygous for a missense mutation of the *Pkdr1* gene, encoding SamCystin, and recapitulate histological features of ADPKD [55,56]. In humans, endogenous VEGF-A levels increase with increasing cyst size, with transcriptome analysis of cysts of different sizes from patients with ADPKD revealing VEGF-A to be upregulated in the largest cysts, containing at least 1 ml of cyst fluid, compared with minimally cystic tissue [57]. The local increase in VEGF-A expression as cystic disease progresses could thus trigger the remodelling of the surrounding peritubular vasculature and their abnormal patterning [25]. Additionally, it has been demonstrated that both VEGFR-1 and VEGFR-2 are expressed by tubular epithelial cell lines and cyst epithelium in rat models of PKD [58–60] indicating that VEGF-A may have an autocrine effect on cyst epithelia. In culture, recombinant VEGF-A leads to the proliferation of proximal tubular cells [58]; an important process in cyst growth [4]. Furthermore, targeted overexpression of *Vegfa* in the kidney tubules of otherwise healthy adult mice causes the formation of cysts [61]. Circulating VEGF-A also increases in patients with ADPKD between 8 and 26 years old; an age where cysts are actively growing but many features of ESKD are not present [62]. The serum VEGF-A levels in these patients correlated with both the total volume of the kidney and cyst volume. There are several molecular pathways implicated in PKD which may trigger the upregulation of VEGF-A in cyst epithelia, including activation of hypoxia-inducible transcription factors [63], angiotensin II [64] and epidermal growth factor [65].

VEGFC signalling in polycystic kidneys

In contrast to VEGF-A, less is known about the expression pattern and function of VEGF-C in either healthy or cystic kidneys. In developing mouse kidneys, VEGF-C is expressed in renal arterioles and interstitial cells, with prominent expression in the renal hilum surrounding VEGFR-3-expressing lymphatics [19]. In healthy adult kidneys, VEGF-C is localised to the smooth muscle cells of renal arterioles, glomerular podocytes and tubular epithelial cells [66,67]. Additionally, in disease contexts, infiltrating macrophages provide a further cellular source of VEGF-C [66,68], which is proposed to be critical for inflammation-induced lymphangiogenesis. VEGFR-3, as well as being expressed by lymphatic vessels in the adult kidney [69], is also detected in fenestrated glomerular and peritubular capillaries [25], akin to expression patterns reported in other high-permeability endothelia in endocrine glands [70]. VEGFR-3 has also been reported to be expressed on kidney macrophages in a unilateral ureteral obstruction model of renal fibrosis in mouse [71]. In PKD, only one study has examined endogenous mRNA levels of *Vegfc* in *Pkd1*$^{n/nl}$ kidneys using quantitative real-time polymerase chain reaction of whole kidney lysates. In this model, *Vegfc* was significantly downregulated in the early stages of disease (at seven days) but then restored to wild-type levels at days 14 and 21 [25]. However, there is currently no information on the cellular localisation of VEGF-C during PKD. It may be postulated that the reduction of endogenous VEGF-C in mouse models of PKD drives the malformation of kidney lymphatics and tortuous, dilated peri-cystic VEGFR-3^{+} blood capillaries [19,25], but this hypothesis requires further investigation.

Modulating vascular endothelial growth factor signalling in PKD

Several studies have now examined if the modulation of vascular endothelial growth signalling might alter the progression of PKD. Initially, inhibition of VEGF-A signalling to VEGFR-1 or VEGFR-2 was the focus of study, with the rationale that preventing the expansion of blood vessels will starve the cysts of the nutrients required for its growth; an approach also exploited in cancer biology [72]. Supporting this hypothesis, the administration of ribozymes targeting either VEGFR-1 or VEGFR-2 in three to eight-week-old *Cy/+* rats led

to a decrease in tubular cell proliferation, which was accompanied by reduced cyst volume and kidney:body weight ratio and an improvement in renal function [60]. VEGFR-1 or VEGFR-2 ribozyme administration led to a decrease in *Vegfr1* and *Vegfr2* mRNA levels respectively, with a reduction of receptor immunoreactivity in tubular epithelium, although VEGF-A levels were not examined in this study. In contrast, administration of an anti-VEGF-A antibody (B20.4.1), which acts on all isoforms of VEGF-A [73], to four-week-old *Cy/+* rats enhanced the proliferation rate of proximal tubular epithelial cells, leading to larger cysts and expansion of the kidney volume [74]. A possible explanation for the discrepancy between these studies is likely the off-target consequences of B20.4.1 administration upon other areas of the kidney, as both *Cy/+* and wild-type rats treated with B20.4.1 displayed glomerular abnormalities and proteinuria [74]. Comparatively, ribozyme administration did not increase albumin to creatinine ratio compared with vehicle-treated *Cy/+* rats.

Recombinant VEGF-C has been shown to have protective effects in mouse models of PKD [25]. Daily intraperitoneal injections of recombinant VEGF-C to seven-day old $Pkd1^{nl/nl}$ mice for two weeks resulted in a halving of kidney:body weight ratio compared with $Pkd1^{nl/nl}$ animals administered vehicle. VEGF-C also improved renal function in $Pkd1^{nl/nl}$ mice, with a lowering of blood urea nitrogen and serum creatinine. Additionally, VEGF-C reduced disease severity in congenital polycystic kidney ($Cys1^{cpk/cpk}$) mice. Though non-orthologous, this model phenocopies the pathology of ARPKD [75]. In $Cys1^{cpk/cpk}$ mice, VEGF-C administration reduced kidney:body weight ratio, average cyst size and extended the lifespan of the animals by one week [25]. These results suggest that VEGF-C delivery is applicable to other models of cystogenesis, although the effects of VEGF-C on slowly progressive mouse models that mimic the temporal progression of human ADPKD has not yet been demonstrated.

There are multiple mechanisms by which VEGF-C might improve PKD (**Figure 3**). Firstly, VEGF-C may act on VEGFR-3-expressing lymphatic capillaries, as VEGF-C delivery

increased the cross-sectional area of kidney lymphatics in *Pkd1*$^{nl/nl}$ mice [25]. The expansion of lymphatics may facilitate the clearance of both excess fluid and inflammatory cells in PKD, concurrent with a reduction in the quantity of renal CD206^{+} inflammatory cells in *Pkd1*$^{nl/nl}$ kidneys following VEGF-C treatment [25]. An alternative yet complementary mechanism of VEGF-C activity may be through its action on VEGFR-3-expressing fenestrated peritubular capillaries. In both *Pkd1*$^{nl/nl}$ and *Cys1*$^{cpk/cpk}$ mice, VEGF-C treatment led to the proliferation of peritubular capillaries and the restoration of the reticular patterning of these vessels that is otherwise lost in mouse models of PKD.

Future directions

From studying the effects of deleting polycystins in endothelium, it is evident that both blood vasculature and lymphatics may have an important role in PKD. However, the mechanisms by which loss of polycystins in the vasculature may contribute to PKD is not yet fully understood. Polycystins may be involved in establishing polarity and migration of endothelial cells during development [34] or involved in intracellular signalling pathways responsible for lymphatic proliferation [42]. Additionally, akin to their ascribed roles in primary cilia, loss of polycystins in endothelial cells may impair the response of these cells to flow and shear-stress, which is fundamental to proper maturation and function of both blood vessels and lymphatics. In support of this, endothelial cells from either *Pkd1* mutant mice or with knock-down of *Pkd2* lose the ability to generate nitric oxide; a key mediator of vascular tone in response to fluid shear stress [39,41].

An attractive prospect is that non-renal vascular abnormalities observed in patients with PKD may result from polycystin loss in the endothelium. Examples of this include impaired flow-mediated dilatation in peripheral arteries of children and young adults or normotensive patients with ADPKD compared with healthy controls [76,77], the association between the mutation position in *PKD1* and cerebrovascular aneurysms [78] and lymphatic malformations

reported in both ADPKD and ARPKD patients [79,80]. Targeted deletion in animal models *in vivo,* complemented by organ-on a chip technologies *in vitro,* may help to tease out whether these clinical observations occur as a consequence of mutations of genes such as *PKD1* in the endothelium, indirectly through *PKD1* loss in vascular smooth muscle [81] or as yet unidentified non-cell-autonomous mechanisms.

Studies in animal models have demonstrated the potential of VEGF therapy for PKD, but there are many unanswered questions which need to be explored prior to translation of this therapeutic strategy towards patient benefit. For a start, the mechanisms by which VEGF-A inhibition and VEGF-C therapy act and the relative contribution of these therapies to different cell types in the polycystic kidney is unknown. For example, to what extent VEGF-C might exert its effects on non-endothelial cell types, such as macrophages [82] or tubular epithelial cells [83] in the context of cystic disease is not clear. These could be teased out by transcriptomic technologies capable of differentiating between cell types, such as single cell RNA-sequencing [84]. A major challenge to surmount will be the development of strategies to direct VEGF therapy to the polycystic kidney. Gene therapy [85], renal-targeted peptides [86] or use of modified growth factors which preferentially bind target tyrosine kinases [87] are potential strategies to enhance therapeutically effect, optimise drug half-life and metabolism and minimise off-target effects and toxicity. There are additionally a multitude of other molecules that could be used to modulate blood or lymphatic endothelia [49,88] which could be explored in the setting of PKD. Regardless of the approach, we envisage the vasculature, as is the case for cancer [89], to develop recognition as a hallmark of PKD. Future therapies targeting the endothelium could eventually be utilised in replacement or in combination with existing compounds targeting the cystic epithelium.

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Figure Legends

Figure 1. Structural features of kidney lymphatics in polycystic kidney disease. Kidney lymphatics in *Pkd1*$^{RC/RC}$ mice, a slow progressive mouse model of autosomal dominant polycystic kidney disease (PKD) were profiled using wholemount immunofluorescence, optical clearing and three-dimensional imaging. At embryonic day 18.5; an early timepoint in this model of PKD, three features were observed. Firstly, lymphatic vessels in the cortex were found in close proximity to corticomedullary epithelial cysts, indicating the potential for solute exchange or molecular crosstalk between lymphatic endothelial cells and cyst epithelium. Secondly, the total number of lymphatic vessel branches relative to kidney volume and the percentage occupancy of the volume of the kidney by lymphatics were significantly reduced in cystic compared to normal kidneys. Finally, the largest caliber lymphatic vessels in the renal hilum had a significantly reduced diameter in cystic disease. Thus, a reduction of kidney lymphatic function, manifesting as impaired drainage of fluid or immune cells, may be a pathological feature of PKD.

Figure 2. Vascular endothelial growth factor signalling in blood endothelial or lymphatic endothelial cells. The top panel shows the ligand-receptor interactions responsible for vascular endothelial growth factor (VEGF) signalling. Whereas VEGF-B exclusively binds to VEGF receptor (R)1, VEGF-A can bind both VEGFR-1 and VEGFR-2. Similarly, VEGF-C and VEGF-D can bind both VEGFR-2 and VEGFR-3. Not shown here are the virally encoded VEGF-E, which binds VEGFR-2, and placental growth factor (PIGF), which binds VEGFR-1. The bottom two panels show the generalised response to stimulation of each VEGF receptor, by their canonical ligands, in either blood endothelial cells (BEC) or lymphatic endothelial cells (LEC).

Figure 3. Potential mechanisms of vascular endothelial growth factor C therapy in polycystic kidney disease. Administration of VEGF-C to mouse models of PKD reduces disease severity. Part of this effect may occur by the upregulation of VEGF-C/VEGFR-3 signalling in lymphatics, promoting the expansion of kidney lymphatics which are otherwise stunted and deformed in PKD. The hyperplasia of lymphatics may increase the efflux of fluid and inflammatory cells that drive cystic disease. An alternative mechanism is through the action of VEGF-C on VEGFR-2^{+} VEGFR-3^{+} peritubular capillaries. VEGF-C stimulates these vessels to proliferate and promotes their remodelling, restoring their architecture. There may be other, as yet unidentified consequences of VEGF-C therapy on cell types within the polycystic kidney, such as through action on immune cells such as macrophages, or through direct activity on cyst epithelial cells.

References

[1] C. Bergmann, L.M. Guay-Woodford, P.C. Harris, S. Horie, D.J.M. Peters, V.E. Torres, Polycystic kidney disease., Nat. Rev. Dis. Primers. 4 (2018) 50. doi:10.1038/s41572-018-0047-y.

[2] S.M. Nauli, F.J. Alenghat, Y. Luo, E. Williams, P. Vassilev, X. Li, et al., Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells., Nat. Genet. 33 (2003) 129–137. doi:10.1038/ng1076.

[3] L.F. Menezes, G.G. Germino, The pathobiology of polycystic kidney disease from a metabolic viewpoint., Nat. Rev. Nephrol. 15 (2019) 735–749. doi:10.1038/s41581-019-0183-y.

[4] K. Ramasubbu, N. Gretz, S. Bachmann, Increased epithelial cell proliferation and abnormal extracellular matrix in rat polycystic kidney disease., J. Am. Soc. Nephrol. 9 (1998) 937–945.

[5] S. Terryn, A. Ho, R. Beauwens, O. Devuyst, Fluid transport and cystogenesis in autosomal dominant polycystic kidney disease., Biochim. Biophys. Acta. 1812 (2011) 1314–1321. doi:10.1016/j.bbadis.2011.01.011.

[6] V.E. Torres, A.B. Chapman, O. Devuyst, R.T. Gansevoort, J.J. Grantham, E. Higashihara, et al., Tolvaptan in patients with autosomal dominant polycystic kidney disease., N. Engl. J. Med. 367 (2012) 2407–2418. doi:10.1056/NEJMoa1205511.

[7] V.E. Torres, A.B. Chapman, O. Devuyst, R.T. Gansevoort, R.D. Perrone, G. Koch, et al., Tolvaptan in Later-Stage Autosomal Dominant Polycystic Kidney Disease., N. Engl. J. Med. 377 (2017) 1930–1942. doi:10.1056/NEJMoa1710030.

[8] J.J. Grantham, Polycystic kidney disease: neoplasia in disguise., Am. J. Kidney Dis. 15 (1990) 110–116. doi:10.1016/s0272-6386(12)80507-5.

[9] J.J. Grantham, Time to treat polycystic kidney diseases like the neoplastic disorders that they are., Kidney Int. 57 (2000) 339–340. doi:10.1046/j.1523-1755.2000.00849.x.

[10] C. Parrot, A. Kurbegovic, G. Yao, M. Couillard, O. Côté, M. Trudel, c-Myc is a regulator of the PKD1 gene and PC1-induced pathogenesis., Hum. Mol. Genet. 28 (2019) 751–763. doi:10.1093/hmg/ddy379.

[11] J.P. Margaria, C.C. Campa, M.C. De Santis, E. Hirsch, I. Franco, The PI3K/Akt/mTOR pathway in polycystic kidney disease: A complex interaction with polycystins and primary cilium., Cell Signal. 66 (2020) 109468. doi:10.1016/j.cellsig.2019.109468.

[12] X. Li, Epigenetics and cell cycle regulation in cystogenesis., Cell Signal. (2019) 109509. doi:10.1016/j.cellsig.2019.109509.

[13] M.I. Parker, A.S. Nikonova, D. Sun, E.A. Golemis, Proliferative signaling by ERBB proteins and RAF/MEK/ERK effectors in polycystic kidney disease., Cell Signal. 67 (2020) 109497. doi:10.1016/j.cellsig.2019.109497.

[14] R.K. Jain, Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy., Science. 307 (2005) 58–62. doi:10.1126/science.1104819.

[15] I. Rowe, M. Chiaravalli, V. Mannella, V. Ulisse, G. Quilici, M. Pema, et al., Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy., Nat. Med. 19 (2013) 488–493. doi:10.1038/nm.3092.

[16] J.L. Huang, A.S. Woolf, D.A. Long, Angiogenesis and autosomal dominant polycystic kidney

disease., Pediatr. Nephrol. 28 (2013) 1749–1755. doi:10.1007/s00467-012-2305-7.

[17] C.P. Le, C.J. Nowell, C. Kim-Fuchs, E. Botteri, J.G. Hiller, H. Ismail, et al., Chronic stress in mice remodels lymph vasculature to promote tumour cell dissemination., Nat. Commun. 7 (2016) 10634. doi:10.1038/ncomms10634.

[18] E. Song, T. Mao, H. Dong, L.S.B. Boisserand, S. Antila, M. Bosenberg, et al., VEGF-C-driven lymphatic drainage enables immunosurveillance of brain tumours., Nature. 577 (2020) 689–694. doi:10.1038/s41586-019-1912-x.

[19] D.J. Jafree, D. Moulding, M. Kolatsi-Joannou, N. Perretta Tejedor, K.L. Price, N.J. Milmoe, et al., Spatiotemporal dynamics and heterogeneity of renal lymphatics in mammalian development and cystic kidney disease., Elife. 8 (2019). doi:10.7554/eLife.48183.

[20] S.J. Dumas, E. Meta, M. Borri, J. Goveia, K. Rohlenova, N.V. Conchinha, et al., Single-Cell RNA Sequencing Reveals Renal Endothelium Heterogeneity and Metabolic Adaptation to Water Deprivation., J. Am. Soc. Nephrol. 31 (2020) 118–138. doi:10.1681/ASN.2019080832.

[21] N. Jourde-Chiche, F. Fakhouri, L. Dou, J. Bellien, S. Burtey, M. Frimat, et al., Endothelium structure and function in kidney health and disease., Nat. Rev. Nephrol. 15 (2019) 87–108. doi:10.1038/s41581-018-0098-z.

[22] R.V. Stan, M. Kubitza, G.E. Palade, PV-1 is a component of the fenestral and stomatal diaphragms in fenestrated endothelia., Proc. Natl. Acad. Sci. USA. 96 (1999) 13203–13207. doi:10.1073/pnas.96.23.13203.

[23] E. Bello-Reuss, K. Holubec, S. Rajaraman, Angiogenesis in autosomal-dominant polycystic kidney disease., Kidney Int. 60 (2001) 37–45. doi:10.1046/j.1523-1755.2001.00768.x.

[24] W. Wei, V. Popov, J.A. Walocha, J. Wen, E. Bello-Reuss, Evidence of angiogenesis and microvascular regression in autosomal-dominant polycystic kidney disease kidneys: a corrosion cast study., Kidney Int. 70 (2006) 1261–1268. doi:10.1038/sj.ki.5001725.

[25] J.L. Huang, A.S. Woolf, M. Kolatsi-Joannou, P. Baluk, R.N. Sandford, D.J.M. Peters, et al., Vascular endothelial growth factor C for polycystic kidney diseases., J. Am. Soc. Nephrol. 27 (2016) 69–77. doi:10.1681/ASN.2014090856.

[26] R. Xu, F. Franchi, B. Miller, J.A. Crane, K.M. Peterson, P.J. Psaltis, et al., Polycystic kidneys have decreased vascular density: a micro-CT study., Microcirculation. 20 (2013) 183–189. doi:10.1111/micc.12022.

[27] O. Ogunlade, J.J. Connell, J.L. Huang, E. Zhang, M.F. Lythgoe, D.A. Long, et al., In vivo three-dimensional photoacoustic imaging of the renal vasculature in preclinical rodent models., Am. J. Physiol. Renal Physiol. 314 (2018) F1145–F1153. doi:10.1152/ajprenal.00337.2017.

[28] K. Alitalo, The lymphatic vasculature in disease., Nat. Med. 17 (2011) 1371–1380. doi:10.1038/nm.2545.

[29] P. Baluk, J. Fuxe, H. Hashizume, T. Romano, E. Lashnits, S. Butz, et al., Functionally specialized junctions between endothelial cells of lymphatic vessels., J. Exp. Med. 204 (2007) 2349–2362. doi:10.1084/jem.20062596.

[30] L.-C. Yao, P. Baluk, R.S. Srinivasan, G. Oliver, D.M. McDonald, Plasticity of button-like junctions in the endothelium of airway lymphatics in development and inflammation., Am. J. Pathol. 180 (2012) 2561–2575. doi:10.1016/j.ajpath.2012.02.019.

[31] R. Gerli, L. Ibba, C. Fruschelli, Ultrastructural cytochemistry of anchoring filaments of human

lymphatic capillaries and their relation to elastic fibers., Lymphology. 24 (1991) 105–112.

[32] L.V. Leak, J.F. Burke, Ultrastructural studies on the lymphatic anchoring filaments., J. Cell Biol. 36 (1968) 129–149. doi:10.1083/jcb.36.1.129.

[33] S.A. Stacker, S.P. Williams, T. Karnezis, R. Shayan, S.B. Fox, M.G. Achen, Lymphangiogenesis and lymphatic vessel remodelling in cancer., Nat. Rev. Cancer. 14 (2014) 159–172. doi:10.1038/nrc3677.

[34] B. Coxam, A. Sabine, N.I. Bower, K.A. Smith, C. Pichol-Thievend, R. Skoczylas, et al., Pkd1 regulates lymphatic vascular morphogenesis during development., Cell Rep. 7 (2014) 623–633. doi:10.1016/j.celrep.2014.03.063.

[35] P. Outeda, D.L. Huso, S.A. Fisher, M.K. Halushka, H. Kim, F. Qian, et al., Polycystin signaling is required for directed endothelial cell migration and lymphatic development., Cell Rep. 7 (2014) 634–644. doi:10.1016/j.celrep.2014.03.064.

[36] K. Hopp, C.J. Ward, C.J. Hommerding, S.H. Nasr, H.-F. Tuan, V.G. Gainullin, et al., Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity., J. Clin. Invest. 122 (2012) 4257–4273. doi:10.1172/JCI64313.

[37] D.J. Jafree, D.A. Long, P.J. Scambler, D. Moulding, Tissue Clearing and Deep Imaging of the Kidney Using Confocal and Two-Photon Microscopy., Methods Mol. Biol. 2067 (2020) 103–126. doi:10.1007/978-1-4939-9841-8_8.

[38] J.J. Grantham, S. Mulamalla, K.I. Swenson-Fields, Why kidneys fail in autosomal dominant polycystic kidney disease., Nat. Rev. Nephrol. 7 (2011) 556–566. doi:10.1038/nrneph.2011.109.

[39] S.M. Nauli, Y. Kawanabe, J.J. Kaminski, W.J. Pearce, D.E. Ingber, J. Zhou, Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1., Circulation. 117 (2008) 1161–1171. doi:10.1161/CIRCULATIONAHA.107.710111.

[40] K. Kim, I. Drummond, O. Ibraghimov-Beskrovnaya, K. Klinger, M.A. Arnaout, Polycystin 1 is required for the structural integrity of blood vessels., Proc. Natl. Acad. Sci. USA. 97 (2000) 1731–1736. doi:10.1073/pnas.040550097.

[41] W.A. AbouAlaiwi, M. Takahashi, B.R. Mell, T.J. Jones, S. Ratnam, R.J. Kolb, et al., Ciliary polycystin-2 is a mechanosensitive calcium channel involved in nitric oxide signaling cascades., Circ. Res. 104 (2009) 860–869. doi:10.1161/CIRCRESAHA.108.192765.

[42] J.-G. Ren, H.-F. Xia, J.-G. Yang, J.-Y. Zhu, W. Zhang, G. Chen, et al., Down-regulation of polycystin in lymphatic malformations: possible role in the proliferation of lymphatic endothelial cells., Hum. Pathol. 65 (2017) 231–238. doi:10.1016/j.humpath.2017.05.016.

[43] M.A. Garcia-Gonzalez, P. Outeda, Q. Zhou, F. Zhou, L.F. Menezes, F. Qian, et al., Pkd1 and Pkd2 are required for normal placental development., PLoS One. 5 (2010). doi:10.1371/journal.pone.0012821.

[44] N. Ferrara, Vascular endothelial growth factor: basic science and clinical progress., Endocr. Rev. 25 (2004) 581–611. doi:10.1210/er.2003-0027.

[45] R.S. Apte, D.S. Chen, N. Ferrara, VEGF in signaling and disease: beyond discovery and development., Cell. 176 (2019) 1248–1264. doi:10.1016/j.cell.2019.01.021.

[46] M.R. Ladomery, S.J. Harper, D.O. Bates, Alternative splicing in angiogenesis: the vascular

endothelial growth factor paradigm., Cancer Lett. 249 (2007) 133–142. doi:10.1016/j.canlet.2006.08.015.

[47] C.M. Failla, M. Carbo, V. Morea, Positive and Negative Regulation of Angiogenesis by Soluble Vascular Endothelial Growth Factor Receptor-1., Int. J. Mol. Sci. 19 (2018). doi:10.3390/ijms19051306.

[48] A. Aspelund, M.R. Robciuc, S. Karaman, T. Makinen, K. Alitalo, Lymphatic system in cardiovascular medicine., Circ. Res. 118 (2016) 515–530. doi:10.1161/CIRCRESAHA.115.306544.

[49] K. Vaahtomeri, S. Karaman, T. Mäkinen, K. Alitalo, Lymphangiogenesis guidance by paracrine and pericellular factors., Genes Dev. 31 (2017) 1615–1634. doi:10.1101/gad.303776.117.

[50] H.M. Bui, D. Enis, M.R. Robciuc, H.J. Nurmi, J. Cohen, M. Chen, et al., Proteolytic activation defines distinct lymphangiogenic mechanisms for VEGFC and VEGFD., J. Clin. Invest. 126 (2016) 2167–2180. doi:10.1172/JCI83967.

[51] V. Joukov, T. Sorsa, V. Kumar, M. Jeltsch, L. Claesson-Welsh, Y. Cao, et al., Proteolytic processing regulates receptor specificity and activity of VEGF-C., EMBO J. 16 (1997) 3898–3911. doi:10.1093/emboj/16.13.3898.

[52] H. Dimke, M.A. Sparks, B.R. Thomson, S. Frische, T.M. Coffman, S.E. Quaggin, Tubulovascular cross-talk by vascular endothelial growth factor a maintains peritubular microvasculature in kidney., J. Am. Soc. Nephrol. 26 (2015) 1027–1038. doi:10.1681/ASN.2014010060.

[53] M. Vitlov Uljević, I. Bočina, I. Restović, N. Kunac, T. Mašek, G. Kretzschmar, et al., Reabsorption in the proximal tubuli-ultrastructural evidence for a novel aspect of renal VEGF trafficking., Cell Tissue Res. 374 (2018) 189–201. doi:10.1007/s00441-018-2850-x.

[54] E. Bello-Reuss, K. Holubec, S. Rajaraman, Angiogenesis in autosomal-dominant polycystic kidney disease., Kidney Int. 60 (2001) 37–45. doi:10.1046/j.1523-1755.2001.00768.x.

[55] K.D. Stringer, R. Komers, S.A. Osman, T.T. Oyama, J.N. Lindsley, S. Anderson, Gender hormones and the progression of experimental polycystic kidney disease., Kidney Int. 68 (2005) 1729–1739. doi:10.1111/j.1523-1755.2005.00589.x.

[56] J.H. Brown, M.-T. Bihoreau, S. Hoffmann, B. Kränzlin, I. Tychinskaya, N. Obermüller, et al., Missense mutation in sterile alpha motif of novel protein SamCystin is associated with polycystic kidney disease in (cy/+) rat., J. Am. Soc. Nephrol. 16 (2005) 3517–3526. doi:10.1681/ASN.2005060601.

[57] X. Song, V. Di Giovanni, N. He, K. Wang, A. Ingram, N.D. Rosenblum, et al., Systems biology of autosomal dominant polycystic kidney disease (ADPKD): computational identification of gene expression pathways and integrated regulatory networks., Hum. Mol. Genet. 18 (2009) 2328–2343. doi:10.1093/hmg/ddp165.

[58] J. Kanellis, S. Fraser, M. Katerelos, D.A. Power, Vascular endothelial growth factor is a survival factor for renal tubular epithelial cells., Am. J. Physiol. Renal Physiol. 278 (2000) F905-15. doi:10.1152/ajprenal.2000.278.6.F905.

[59] G. Villegas, B. Lange-Sperandio, A. Tufro, Autocrine and paracrine functions of vascular endothelial growth factor (VEGF) in renal tubular epithelial cells., Kidney Int. 67 (2005) 449–457. doi:10.1111/j.1523-1755.2005.67101.x.

[60] Y. Tao, J. Kim, Y. Yin, I. Zafar, S. Falk, Z. He, et al., VEGF receptor inhibition slows the progression of polycystic kidney disease., Kidney Int. 72 (2007) 1358–1366. doi:10.1038/sj.ki.5002550.

[61] S. Hakroush, M.J. Moeller, F. Theilig, B. Kaissling, T.P. Sijmonsma, M. Jugold, et al., Effects of increased renal tubular vascular endothelial growth factor (VEGF) on fibrosis, cyst formation, and glomerular disease., Am. J. Pathol. 175 (2009) 1883–1895. doi:10.2353/ajpath.2009.080792.

[62] B.Y. Reed, A. Masoumi, E. Elhassan, K. McFann, M.A. Cadnapaphornchai, D.M. Maahs, et al., Angiogenic growth factors correlate with disease severity in young patients with autosomal dominant polycystic kidney disease., Kidney Int. 79 (2011) 128–134. doi:10.1038/ki.2010.355.

[63] A. Hofherr, T. Busch, M. Köttgen, HIF-1α drives cyst growth in advanced stages of autosomal dominant polycystic kidney disease., Kidney Int. 94 (2018) 849–851. doi:10.1016/j.kint.2018.07.012.

[64] D. Feliers, Y. Gorin, G. Ghosh-Choudhury, H.E. Abboud, B.S. Kasinath, Angiotensin II stimulation of VEGF mRNA translation requires production of reactive oxygen species., Am. J. Physiol. Renal Physiol. 290 (2006) F927-36. doi:10.1152/ajprenal.00331.2005.

[65] D. Zepeda-Orozco, H.M. Wen, B.A. Hamilton, N.S. Raikwar, C.P. Thomas, EGF regulation of proximal tubule cell proliferation and VEGF-A secretion., Physiol. Rep. 5 (2017). doi:10.14814/phy2.13453.

[66] K. Matsui, K. Nagy-Bojarsky, P. Laakkonen, S. Krieger, K. Mechtler, S. Uchida, et al., Lymphatic microvessels in the rat remnant kidney model of renal fibrosis: aminopeptidase p and podoplanin are discriminatory markers for endothelial cells of blood and lymphatic vessels., J. Am. Soc. Nephrol. 14 (2003) 1981–1989. doi:10.1097/01.asn.0000076078.50889.43.

[67] R.R. Foster, S.C. Satchell, J. Seckley, M.S. Emmett, K. Joory, C.Y. Xing, et al., VEGF-C promotes survival in podocytes., Am. J. Physiol. Renal Physiol. 291 (2006) F196-207. doi:10.1152/ajprenal.00431.2005.

[68] Y.-C. Guo, M. Zhang, F.-X. Wang, G.-C. Pei, F. Sun, Y. Zhang, et al., Macrophages Regulate Unilateral Ureteral Obstruction-Induced Renal Lymphangiogenesis through C-C Motif Chemokine Receptor 2-Dependent Phosphatidylinositol 3-Kinase-AKT-Mechanistic Target of Rapamycin Signaling and Hypoxia-Inducible Factor-1α/Vascular Endothelial Growth Factor-C Expression., Am. J. Pathol. 187 (2017) 1736–1749. doi:10.1016/j.ajpath.2017.04.007.

[69] S. Yazdani, F. Poosti, A.B. Kramer, K. Mirković, A.J. Kwakernaak, M. Hovingh, et al., Proteinuria triggers renal lymphangiogenesis prior to the development of interstitial fibrosis., PLoS One. 7 (2012) e50209. doi:10.1371/journal.pone.0050209.

[70] T.A. Partanen, J. Arola, A. Saaristo, L. Jussila, A. Ora, M. Miettinen, et al., VEGF-C and VEGF-D expression in neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues., FASEB J. 14 (2000) 2087–2096. doi:10.1096/fj.99-1049com.

[71] C. Zhang, Y. Zhang, C. Zhang, Y. Liu, Y. Liu, G. Xu, Pioglitazone increases VEGFR3 expression and promotes activation of M2 macrophages via the peroxisome proliferator-activated receptor γ., Mol. Med. Rep. 19 (2019) 2740–2748. doi:10.3892/mmr.2019.9945.

[72] R.K. Jain, Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia., Cancer Cell. 26 (2014) 605–622. doi:10.1016/j.ccell.2014.10.006.

[73] W.-C. Liang, X. Wu, F.V. Peale, C.V. Lee, Y.G. Meng, J. Gutierrez, et al., Cross-species vascular endothelial growth factor (VEGF)-blocking antibodies completely inhibit the growth of human tumor xenografts and measure the contribution of stromal VEGF., J. Biol. Chem. 281 (2006) 951–961. doi:10.1074/jbc.M508199200.

[74] S. Raina, M. Honer, S.D. Krämer, Y. Liu, X. Wang, S. Segerer, et al., Anti-VEGF antibody treatment accelerates polycystic kidney disease., Am. J. Physiol. Renal Physiol. 301 (2011) F773-83. doi:10.1152/ajprenal.00058.2011.

[75] X. Hou, M. Mrug, B.K. Yoder, E.J. Lefkowitz, G. Kremmidiotis, P. D'Eustachio, et al., Cystin, a novel cilia-associated protein, is disrupted in the cpk mouse model of polycystic kidney disease., J. Clin. Invest. 109 (2002) 533–540. doi:10.1172/JCI14099.

[76] K.L. Nowak, H. Farmer, M.A. Cadnapaphornchai, B. Gitomer, M. Chonchol, Vascular dysfunction in children and young adults with autosomal dominant polycystic kidney disease., Nephrol. Dial. Transplant. 32 (2017) 342–347. doi:10.1093/ndt/gfw013.

[77] A. Lorthioir, R. Joannidès, I. Rémy-Jouet, C. Fréguin-Bouilland, M. Iacob, C. Roche, et al., Polycystin deficiency induces dopamine-reversible alterations in flow-mediated dilatation and vascular nitric oxide release in humans., Kidney Int. 87 (2015) 465–472. doi:10.1038/ki.2014.241.

[78] S. Rossetti, D. Chauveau, V. Kubly, J.M. Slezak, A.K. Saggar-Malik, Y. Pei, et al., Association of mutation position in polycystic kidney disease 1 (PKD1) gene and development of a vascular phenotype., Lancet. 361 (2003) 2196–2201. doi:10.1016/S0140-6736(03)13773-7.

[79] A.A. Kashgari, N. Ozair, A. Al Zahrani, M.O. Al Otibi, K. Al Fakeeh, Renal lymphangiomatosis, a rare differential diagnosis for autosomal recessive polycystic kidney disease in pediatric patients., Radiol. Case Rep. 12 (2017) 70–72. doi:10.1016/j.radcr.2016.11.016.

[80] N.D. Thimmappa, J.D. Blumenfeld, M.A.C. Cerilles, A. Dunning, S.L. Donahue, W.O. Bobb, et al., Cisterna chyli in autosomal dominant polycystic kidney disease., J. Magn. Reson. Imaging. 41 (2015) 142–148. doi:10.1002/jmri.24527.

[81] S. Hassane, N. Claij, M. Jodar, A. Dedman, I. Lauritzen, F. Duprat, et al., Pkd1-inactivation in vascular smooth muscle cells and adaptation to hypertension., Lab. Invest. 91 (2011) 24–32. doi:10.1038/labinvest.2010.159.

[82] S. D'Alessio, C. Correale, C. Tacconi, A. Gandelli, G. Pietrogrande, S. Vetrano, et al., VEGF-C-dependent stimulation of lymphatic function ameliorates experimental inflammatory bowel disease., J. Clin. Invest. 124 (2014) 3863–3878. doi:10.1172/JCI72189.

[83] X. Chang, Q. Yang, C. Zhang, Y. Zhang, X. Liang, Y. Liu, et al., Roles for VEGF-C/NRP-2 axis in regulating renal tubular epithelial cell survival and autophagy during serum deprivation., Cell Biochem. Funct. 37 (2019) 290–300. doi:10.1002/cbf.3402.

[84] H. Wu, B.D. Humphreys, The promise of single-cell RNA sequencing for kidney disease investigation., Kidney Int. 92 (2017) 1334–1342. doi:10.1016/j.kint.2017.06.033.

[85] Y.-S. Yoon, T. Murayama, E. Gravereaux, T. Tkebuchava, M. Silver, C. Curry, et al., VEGF-C gene therapy augments postnatal lymphangiogenesis and ameliorates secondary lymphedema., J. Clin. Invest. 111 (2003) 717–725. doi:10.1172/JCI15830.

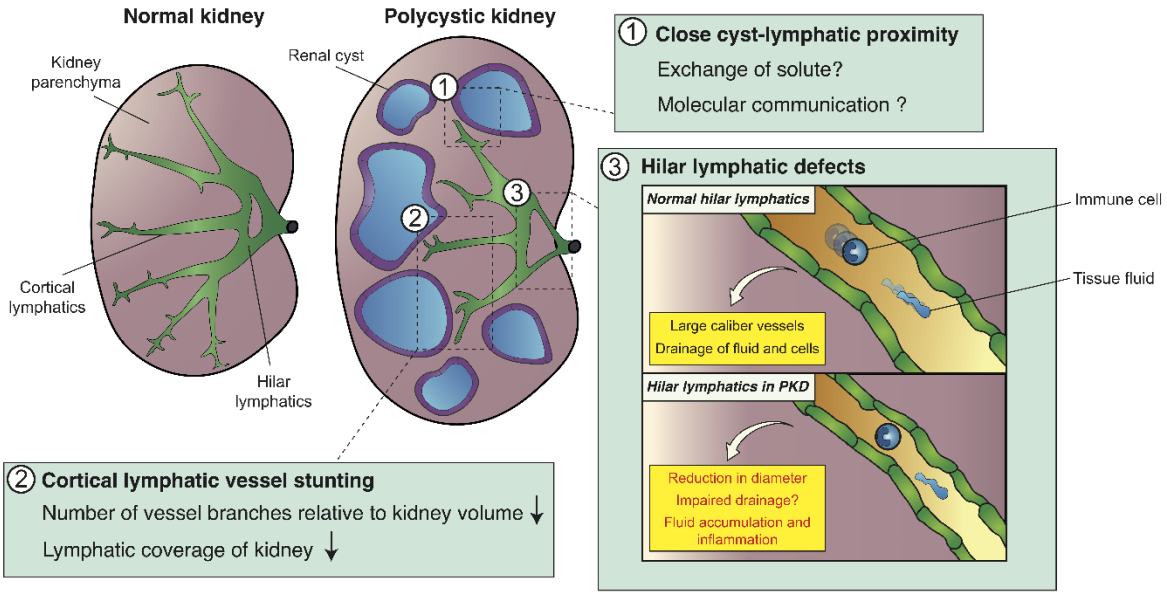
[86] G.L. Bidwell, F. Mahdi, Q. Shao, O.C. Logue, J.P. Waller, C. Reese, et al., A kidney-selective biopolymer for targeted drug delivery., Am. J. Physiol. Renal Physiol. 312 (2017) F54–F64.

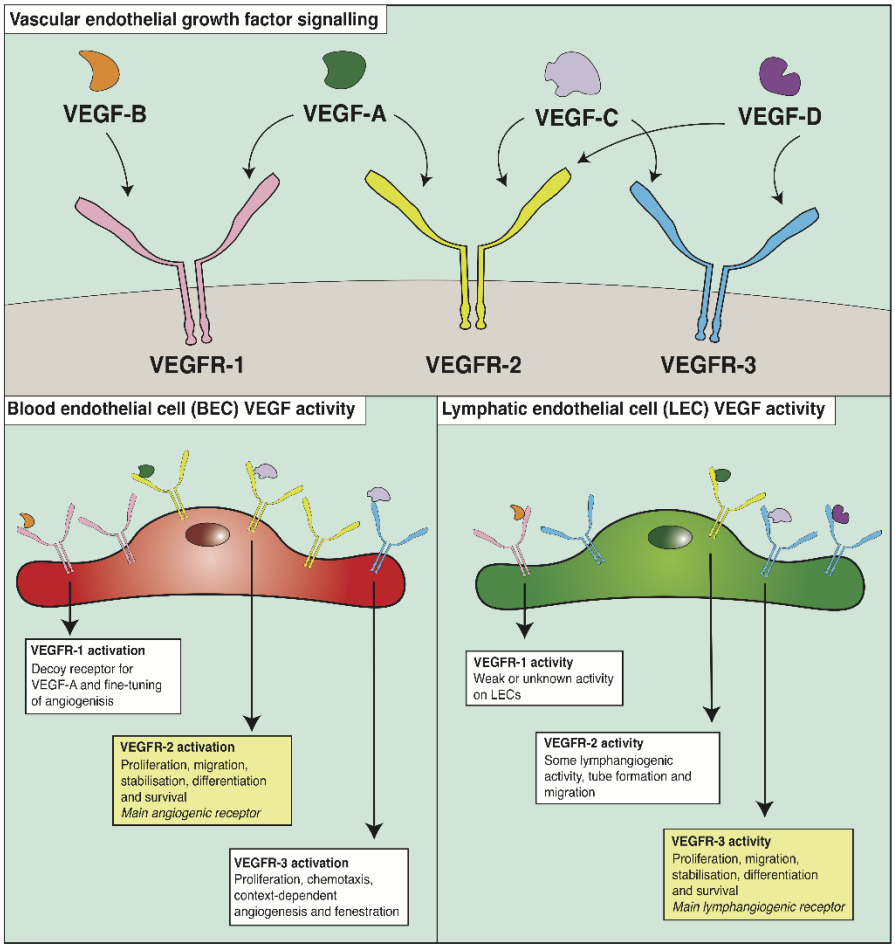
doi:10.1152/ajprenal.00143.2016.

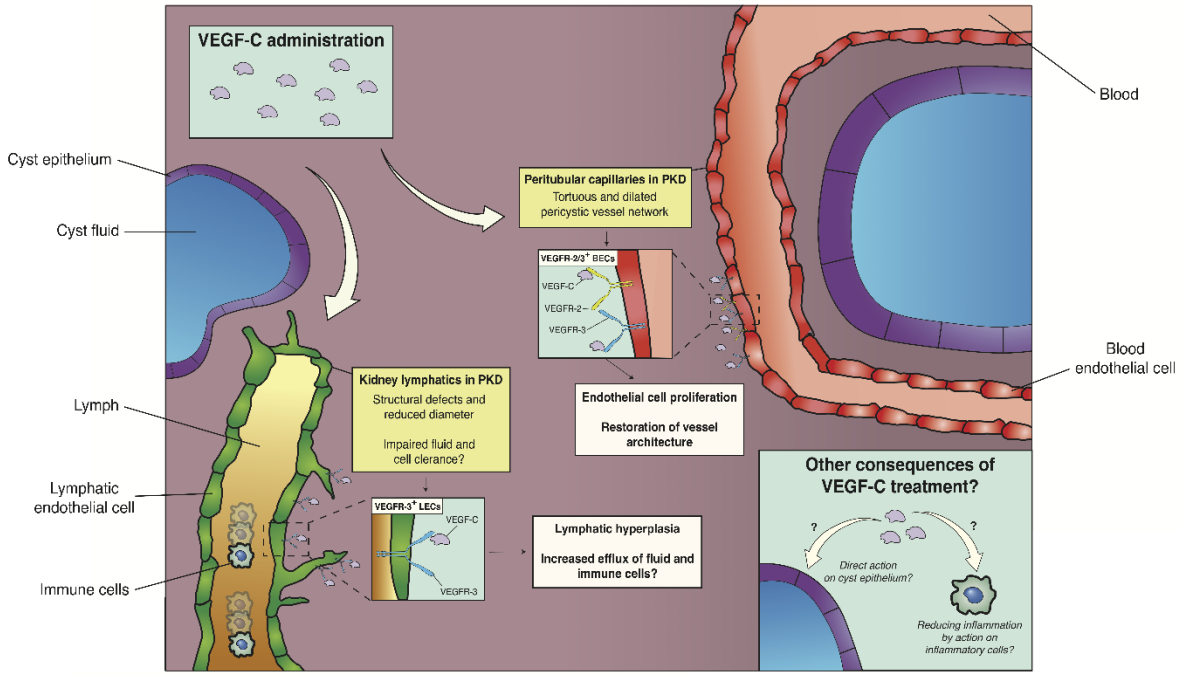
[87] V. Joukov, V. Kumar, T. Sorsa, E. Arighi, H. Weich, O. Saksela, et al., A recombinant mutant vascular endothelial growth factor-C that has lost vascular endothelial growth factor receptor-2 binding, activation, and vascular permeability activities., J. Biol. Chem. 273 (1998) 6599–6602. doi:10.1074/jbc.273.12.6599.

[88] D.A. Long, J.T. Norman, L.G. Fine, Restoring the renal microvasculature to treat chronic kidney disease., Nat. Rev. Nephrol. 8 (2012) 244–250. doi:10.1038/nrneph.2011.219.

[89] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation., Cell. 144 (2011) 646–674. doi:10.1016/j.cell.2011.02.013.


Normal kidney
Polycystic kidney
Kidney parenchyma
Renal cyst
Cortical lymphatics
Hilar lymphatics
1
2
3
① Close cyst-lymphatic proximity
Exchange of solute?
Molecular communication ?
③ Hilar lymphatic defects
Normal hilar lymphatics
Immune cell
Tissue fluid
Large caliber vessels
Drainage of fluid and cells
Hilar lymphatics in PKD
Reduction in diameter
Impaired drainage?
Fluid accumulation and inflammation
② Cortical lymphatic vessel stunting
Number of vessel branches relative to kidney volume ↓
Lymphatic coverage of kidney ↓


Vascular endothelial growth factor signalling
VEGF-B
VEGF-A
VEGF-C
VEGF-D
VEGFR-1
VEGFR-2
VEGFR-3
Blood endothelial cell (BEC) VEGF activity
VEGFR-1 activation
Decoy receptor for VEGF-A and fine-tuning of angiogenisis
VEGFR-2 activation
Proliferation, migration, stabilisation, differentiation and survival
Main angiogenic receptor
VEGFR-3 activation
Proliferation, chemotaxis, context-dependent angiogenesis and fenestration
Lymphatic endothelial cell (LEC) VEGF activity
VEGFR-1 activity
Weak or unknown activity on LECs
VEGFR-2 activity
Some lymphangiogenic activity, tube formation and migration
VEGFR-3 activity
Proliferation, migration, stabilisation, differentiation and survival
Main lymphangiogenic receptor


VEGF-C administration
Cyst epithelium
Cyst fluid
Lymph
Lymphatic endothelial cell
Immune cells
Blood
Blood endothelial cell
Peritubular capillaries in PKD
Tortuous and dilated pericystic vessel network
VEGFR-2/3+ BECs
VEGF-C
VEGFR-2
VEGFR-3
Endothelial cell proliferation
Restoration of vessel architecture
Kidney lymphatics in PKD
Structural defects and reduced diameter
Impaired fluid and cell clearance?
VEGFR-3+ LECs
VEGF-C
VEGFR-3
Lymphatic hyperplasia
Increased efflux of fluid and immune cells?
Other consequences of VEGF-C treatment?
?
?
Direct action on cyst epithelium?
Reducing inflammation by action on inflammatory cells?