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Role of VEGF in Kidney Development, Microvascular Maintenance and Pathophysiology of Renal Disease

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Vascular endothelial growth factor, VEGF, is essential for endothelial cell differentiation (vasculogenesis) and for the sprouting of new capillaries from preexisting vessels (angiogenesis). In addition, there is strong evidence that VEGF is a survival factor allowing the cells to survive and proliferate under conditions of extreme stress.

Hypoxia is a key regulator of VEGF gene expression. Besides hypoxia, many cytokines, hormones and growth factors can up-regulate VEGF mRNA expression in various cell types.

VEGF is present in the glomerulus of both the fetal and adult kidney. The VEGF produced by glomerular epithelial cell may be responsible for maintenance of the fenestrated phenotype of glomerular epithelial cells, thus facilitating the high rate of glomerular ultrafiltration. But there is little known about the role of VEGF in the tubule.

VEGF is thought to be involved in many kinds of kidney diseases. Whereas VEGF has a beneficial role in the pathogenesis in some diseases, it does harmful action in others. Because VEGF is known to be associated with the pathogenesis of some diseases, such as diabetic nephropathy, renal tumor and polycystic kidney disease, the study about the role of VEGF is going to be a target for disease control. On the other hand, an attempt at enhancing the role of VEGF has to be made at diseases like several ARF models and experimental glomerulonephritis.

Key Words : VEGF, Kidney, Disease

INTRODUCTION

Vascular endothelial growth factor, VEGF, also termed vascular permeability factor, VPF, has been at the center stage of vascular research for the past 2 decades. These years witnessed the expansion of the VEGF family of proteins, elucidation of their functions and attempts at utilizing these proteins and their respective neutralizing antibodies for therapeutic purposes, both in experimental animals and in clinical trials of myocardial and limb revascularization.

Angiogenesis is essential not only for organ development and differentiation during embryogenesis but also for physiological situations, such as wound healing and reproductive functions[1]. Vascular supply is also thought to be implicated in the pathogenesis of a variety of disorders, including proliferative retinopathies, age-related macular degeneration, tumors, rheumatoid arthritis and psoriasis. Vascular endothelial growth factor (VEGF) is essential for endothelial cell differentiation (vasculogenesis) and for the sprouting of new capillaries from preexisting vessels (angiogenesis)[2, 3]. In addition, there is also strong evidence that VEGF is a survival factor allowing the cells to survive and proliferate under conditions of extreme stress, both in vitro and in vivo[4-8]. This review will focus on the latest developments in understanding the role of VEGF in renal development, function and dysfunction.

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VEGF family members and their receptors

VEGF is a distant relative of platelet-derived growth factor (PDGF) and is a member of a family of related growth factors that now includes VEGF-B, -C, -D and -E and placenta growth factor (PlGF)[9]. In particular, VEGF, referred to also as VEGF-A, is a major regulator of normal and abnormal angiogenesis.

Alternative splicing of human VEGF mRNA from a single gene containing eight exons[10] gives rise to at least five different isoforms of 121, 145, 165, 189 and 206 amino acid residues[7, 8, 11]. Mouse and rat VEGF isoforms are shorter by one amino acid[12, 13]. $VEGF_{121}$ fails to bind to heparin, while $VEGF_{165}$ is a basic, heparin-binding protein. $VEGF_{189}$ and $VEGF_{206}$ are more basic and bind to heparin with ever greater affinity than $VEGF_{165}$[14]. $VEGF_{121}$, $VEGF_{145}$ and $VEGF_{165}$ are secreted and readily diffusible. In contrast, $VEGF_{189}$ and $VEGF_{206}$ are almost completely sequestrated in the extracellular matrix (ECM)[15]. Human $VEGF_{165}$ is typically expressed as a 46-kDa homodimer of 23-kDa monomers and is the most abundant and the most biologically active form.

Two distant receptor tyrosine kinases (RTKs) have been identified for VEGF, VEGF-R1 (Flt-1) and VEGF-R2 (KDR/Flk-1), which share ~44% amino acid homology with each other[16, 17]. A third receptor, VEGF-R3 (Flt-4), binds VEGF-C and -D and does not bind VEGF-A[18, 19]. PlGF and VEGF-B bind with high affinity only to VEGF-R1 and do not bind to VEGF-R2. VEGF-E binds with high affinity to VEGF- R2 (Table 1).

Neuropilin-1 (NP-1) was recently identified as a new receptor for VEGF. NP-1, previously known as a neuronal receptor, was shown to function also in endothelial cells as an isoform-specific receptor for $VEGF_{165}$ and as a co-receptor in vitro of VEGF R2[20].

Table 1. Binding of VEGF family members to the known VEGF tyrosine kinase receptors

	VEGF R1 (Flt-1)	VEGFR2 (KDR/Flk-1)	VEGF R3 (Flt-4)
VEGF-A	Yes	Yes	No
VEGF-B	Yes	No	No
VEGF-C	No	Yes	Yes
VEGF-D	No	Yes	Yes
VEGF-F	No	Yes	No
PlGF	Yes	No	No

Regulation of VEGF gene expression

Hypoxia

Hypoxia is a key regulator of VEGF gene expression, both in vitro and in vivo. Hypoxia induces transcription of the VEGF gene[21, 22]. In the VEGF gene, a 28 bp hypoxia response element (HRE) is located approximately 1 kb upstream of the transcription initiation site[23]. Transcription activation of the VEGF gene is mediated by binding of the hypoxia-inducible factor-1 (HIF-1α/β) to this element in hypoxic condition. The HIF-1α subunit is the $_pO_2$-sensitive partner[24].

However, transcriptional activation is not the only mechanism leading to VEGF up-regulation in response to hypoxia. Increased mRNA stability is an important post- transcriptional component[25, 26]. Hypoxia induces transcription of VEGF gene and stabilization of VEGF mRNA.

Hormones and cytokines

Many cytokines, hormones and growth factors can up-regulate VEGF mRNA expression in various cell types. Epidermal growth factor, TGF-β or keratinocyte growth factor result in a marked induction of VEGF gene expression[27]. Both interleukin 1α and prostaglandin E2 induce expression of VEGF in cultured synovial fibroblasts, suggesting the participation of such inductive mechanisms in inflammatory angiogenesis[28]. Insulin-like growth factor 1 has also been shown to induce VEGF mRNA and protein in cultured colorectal carcinoma cells[29]. Thyroid-stimulating hormones and ACTH are able to induce VEGF gene expression in vitro[30, 31].

VEGF is up-regulated by renin and angiotensin II[32, 33]. In turn, VEGF is also known to stimulate expression of angiotensin-converting enzyme and production of angiotensin II[34, 35]. Although there were many reports showing that angiotensin II is a potent stimulator of VEGF expression in several cell lines[32, 33, 36], one study showed the opposite relations between angiotensin II and VEGF. According to this latter study, angiotensin II is a potent inhibitor of VEGF expression in mTAL cells[37].

Distribution of VEGF and its receptors in the kidney

VEGF is present in the glomerulus of both the fetal and adult kidney. By immunohistochemistry of the human kidney, VEGF is expressed in the glomerular matrix and in the glomerular cells of both the fetal and adult human kidneys[38].

In the adult rat kidney, VEGF is constitutively expressed in glomerular podocytes. In addition, consistent with the fact that glomerular epithelial cells (GEC) have been identified as the site of constitutive production of VEGF[39], several reports showed that VEGF is present in the glomerulus of the kidney[40, 41].

In the tubule, so far, VEGF expression in the normal human kidney has been known to be confined to the distal and collecting duct epithelium[38]. The expression of VEGF protein in the distal tubule was also found in the fetal kidney. VEGF expression in the distal tubule was weaker than in the glomerulus[41].

In the rat, normal kidney showed diffuse expression of VEGF in all tubules of the renal cortex and medulla[42]. Proximal tubules in the human kidney exhibit only faint, if any, labeling of VEGF mRNA and VEGF protein when studied by in situ hybridization and immunohistochemistry[39, 42]. But, recently, some publications reported that human proximal tubular cells produced VEGF in culture[43, 44].

VEGF receptors are predominantly expressed in the glomerular capillaries[45]. To a lesser degree, VEGF receptors are found in other capillaries and veins by immunohistochemistry. In the fetal kidney, glomeruli demonstrated strong labeling for flt-1 and KDR mRNA by in situ hybridization with antisense riboprobes[38]. Besides endothelial cells, other cells, such as mesangial cells[46] and tubular cells[47], function as effectosr of VEGF, although the precise actions on these cells remain unclear.

Functional role of VEGF in the glomerulus and tubule of the kidney

The alternative exon splicing of a single VEGF gene gives rise to 5 isoforms of VEGF. The major known functional difference among the various VEGF isoforms is their ability to bind heparin and heparan sulfate proteoglycans distributed on a cellular surface and within the extracellular matrix and basement membrane.

The fact that the glomerular epithelial cell (GEC) expresses mRNA for the soluble secretory form VEGF-120 and for the soluble matrix-associable VEGF-164 and insoluble, heparin-binding matrix-associated VEGF-188 and VEGF-205 suggests that the glomerular basement membrane may be the site of VEGF accumulation and storage. Thus we hypothesized that VEGF produced by GEC is stored at the basement membrane and released to the endothelial cells, thus effectively completing the loop of paracrine action.

VEGF is constitutively expressed in the normal kidney where it is primarily localized to glomerular podocytes and tubular cells. Furthermore, the expression of VEGF receptors on the endothelial cells as an effector system suggests that VEGF effects may be mediated via its binding to heparan sulfate proteoglycans, which act to shuttle VEGF across the basement membrane[48].

It has been suggested that VEGF produced by the glomerular epithelial cell (GEC) may be responsible for maintenance of the fenestrated phenotype of glomerular epithelial cells[38, 39], thus facilitating the high rate of glomerular ultrafiltration. Along these lines, we showed that VEGF acts on the permeability of endothelial cell monolayers in vitro. Caveolae, the plausible structures involved in the increase in endothelial permeability[49], became organized into elongated cell-spanning structures in endothelial cells shortly after exposure to VEGF. These structures were permeable to high molecular weight dextran. Two days after addition of VEGF, endothelial cells exhibited diaphragmed fenestrae[50]. Thus, it is conceivable that VEGF elicits a rapid increase in vascular permeability via mobilization of caveolae and formation of cell-spanning channels, whereas the long-term effect requires formation of fenestra (Figure 1).

In contrast to the glomerulus, little is known about the role of VEGF in the tubule. In the kidney, the peculiar anatomy of microvasculature is characterized by the first division of arterioles into glomerular capillaries, which is followed by the "rete mirabile" of the second subdivision of the efferent arterioles into peritubular capillaries. Both the glomerular and peritubular capillary endothelium are characterized by extensive fenestration and high permeability to solutes. This analogy with the microvasculature of endocrine organs and tumors provided an impetus for investigation into the production of VEGF in the different nephron segments of the kidney[51].

In the remnant kidney model, Kang et al.[37] observed a strong correlation between the number of peritubular capillaries and tubular VEGF expressions. Recently, we investigated the production and role of VEGF in the proximal tubular and glomerular epithelial cells by using epithelial-endothelial cell co-cultures[44]. Our study showed that proximal tubular epithelial cells constitutively produce VEGF and VEGF secreted by proximal tubular epithelial cells supports angiogenesis in culture. Furthermore, VEGF production is dramatically up-regulated by hypoxia and by high glucose. However, the angiogenic response in vitro is limited to the hypoxic condition only and does not occur under hyperglycemia. These data indicate that, while VEGF is necessary for angiogenesis, it is not sufficient under conditions when endothelial cells become dysfunctional, like in hyperglycemia. Collectively, we hypothesize that the regulated production of VEGF by the proximal tubular epithelium may play a profound role in the regulation of the peritubular capillary network (Figure 2).

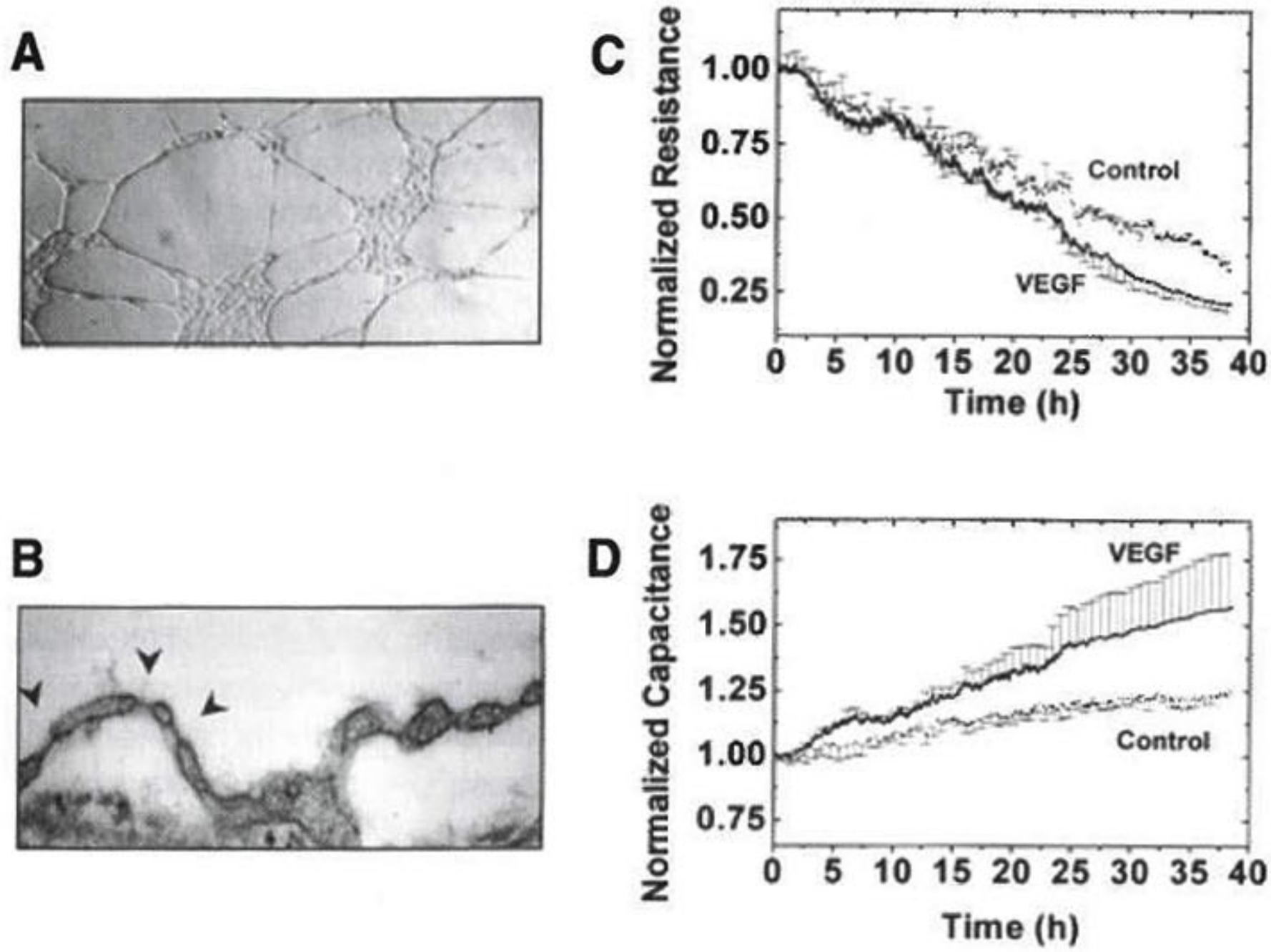

Figure 1. Long-term effect of VEGF resulted in the formation of fenestration of endothelial cells. (A) HUVEC were cultured on matrigel for 36 h in the presence of 10 ng/mL VEGF-165. Light microscopic image shows an elaborate capillary-like network formed under these conditions. (B) Transmission electron micrograph shows diaphragmed fenestrae (arrowheads). (C) and (D) changes in electrical resistance and capacitance, respectively, in HUVEC treated with 10 ng/mL VEGF-165 (control cells were deprived of VEGF), demonstrating concomitant decrease in resistance and increase in capacitance compared with control. One-way ANOVA of experimental and control data in B and C showed that these curves are significantly different at $p<0.05$. HUVEC: human umbilical endothelial cell. Reprinted with permission from ref. 50.

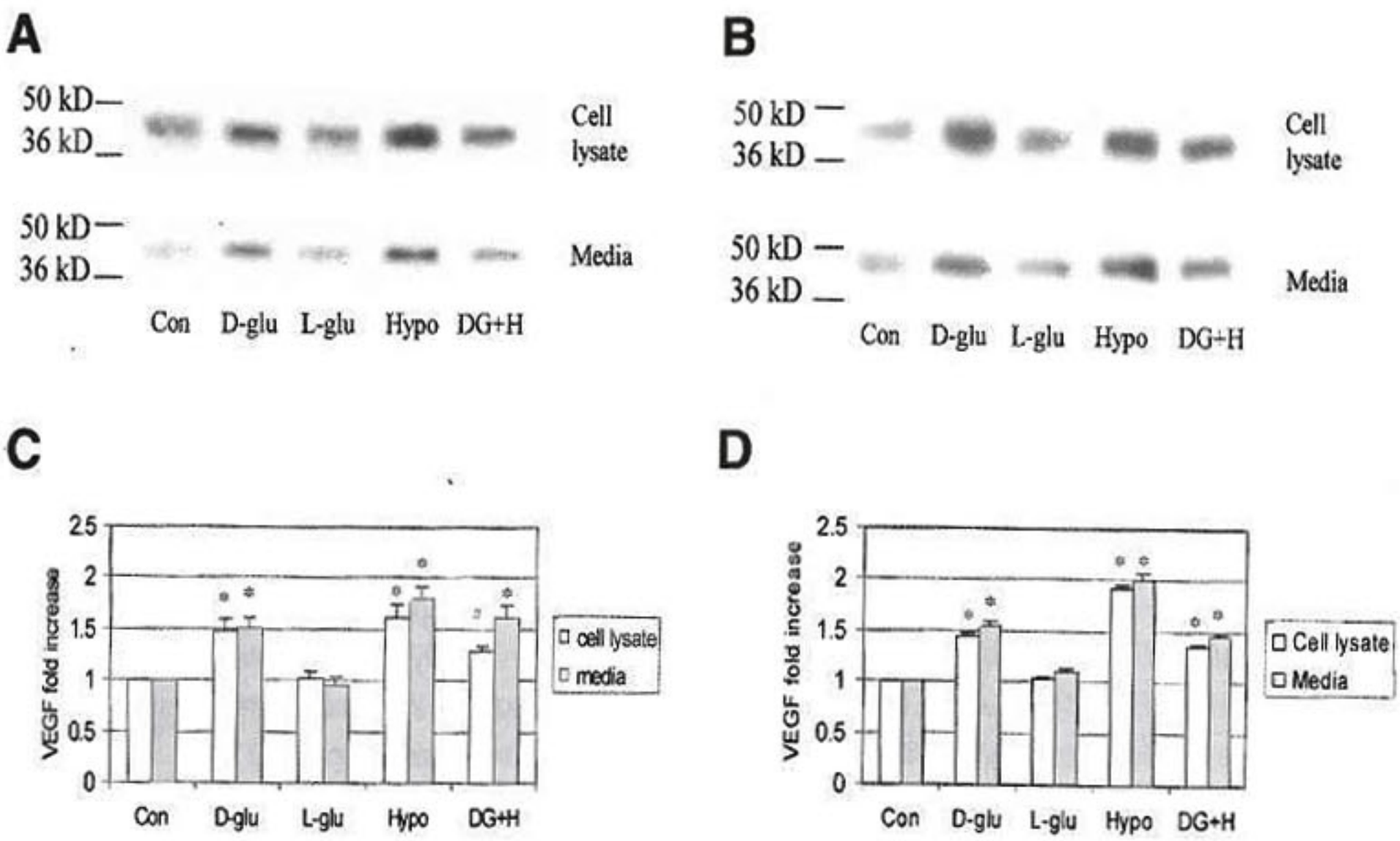

Figure 2. VEGF protein expression in GEC and RPTEC (A and B). Immunoprecipitation for VEGF in GEC (A) and RPTEC (B). Both bands shown are VEGF protein expressions from cell lysate and media, respectively. VEGF was detected using immunoprecipitation with polyclonal antibody followed by blotting with the monoclonal antibody. (C and D) VEGF expression in GEC (C) and RPTEC (D).
GEC: rat glomerular epithelial cell, RPTEC: human renal proximal tubular epithelial cell; Con: control; D-glu: 30 mM D-glucose for 24 hours; L-glu: 30 mM L-glucose for 24 hours; Hypo: hypoxia for 24 hours; DG+H: 30 mM D-glucose+hypoxia for 24 hours.
*: $p<0.01$ compared with the control group, #: $p<0.05$ compared with the control group. Reprinted with permission from ref. 44.

VEGF in renal diseases

1. Acute renal failure

Hypoxia is a well-established potent stimulus for VEGF production in some cells, acting by inducing transcription of VEGF gene and stabilization of VEGF mRNA[21, 26, 52]. We also observed that VEGF production was increased after hypoxic injury in the glomerular (GEC) and human renal proximal tubular epithelial cells (RPTEC) in culture[44]. Interestingly, a 24-hour hypoxia resulted in a 2.4- and 4.9-fold increase in VEGF mRNA in GEC and RPTEC, respectively, indicating that VEGF production after hypoxia is more robust in the tubular cells than in the glomerulus.

The most susceptible region to hypoxia is the outer medulla[53]. The tubules in this region are normally in a borderline hypoxic state due to the countercurrent circulation and high oxygen demand of the medullary thick ascending tubule and the S3 segments of the proximal tubule. Thus, hypoxia can induce tubular and interstitial cell injury, cell activation, proliferation, cytokine generation and matrix synthesis associated with an increased expression of HIF-1α[54, 55].

There is strong evidence that VEGF is a survival factor for endothelial cells. VEGF acts as a survival factor by preventing endothelial cell apoptosis via stimulation of VEGF R-2-mediated signaling cascade involving PI3K/Akt pathway[6, 7]. This would be an obvious teleologic justification for tubular secretion of VEGF, since survival of a tubular cell during hypoxic injury is likely to be critically dependent on the survival of the adjacent endothelium and maintenance of the peritubular blood flow.

One study suggests that the change of VEGF distribution, rather than an increase in VEGF production, is responsible for the response to hypoxic injury. The authors show that acute re-distribution of cytoplasmic VEGF to the basolateral aspect of tubular epithelial cells in response to hypoxic injury[42] can protect endothelial cells.

Besides ischemic injury, VEGF accelerates renal recovery in toxin-induced ARF[56]. VEGF resulted in greater recovery of microvasculature and better renal function in the TMA (thrombotic microangiopathy) animal model induced by the anti-glomerular endothelial cell IgG.

One study shows that peritubular capillary loss is associated with chronic tubulointerstitial injury (CTI) and the pattern of VEGF expression has been changed[57]. VEGF expression was increased in the tubule, especially in morphologically intact or hypertrophic ones. This supports the postulate on the role of VEGF in chronic ischemic injury.

2. Glomerular diseases

The patterns of VEGF expression in glomerular diseases are complicated. VEGF has been implicated in the induction of proteinuria in renal disease[58]. In addition, the up-regulation of VEGF expression in the mesangial cells during mesangio-proliferative disease was described[59].

Recently, one study showed that the addition of VEGF to cultured human mesangial cells induced their proliferation and that VEGF receptors are present in human mesangial cells[46]. Mesangial cell proliferation is a common response of the glomerulus to diverse injuries in both human and experimental glomerular disease, and several growth factors have been implicated in this process. There are several potential sources of glomerular VEGF. VEGF can be released by glomerular cells and by infiltrating T lymphocytes[60] or monocytes[61]. Among resident glomerular cells, epithelial[39] and mesangial cells[61] produce VEGF in vitro. VEGF production is up- regulated by several growth factors, inflammatory cytokines and vasoactive agents in the kidney. Thus, a series of stimuli involved in glomerular injury can induce both VEGF and modulate the expression of its receptor.

On the other hand, many studies have shown the beneficial role of VEGF in the pathogenesis of glomerular diseases. In the experimental anti-Thy-1.1 glomerulonephritis, VEGF may participate in the healing of glomerular lesions[62]. Another study showed that systemic administration of VEGF induced glomerular repair and resolution of glomerulonephritis in the experimentally induced glomerulonephritis[63]. The authors discovered that the recovery was associated with stimulation of angiogenesis, vascular remodeling and angiogenic capillary repair, all playing an important role in recovery from severe glomerular damage. Different from one report as mentioned above[46], proliferation and/or activation of mesangial cells, even in rats treated with VEGF, was significantly decreased compared to the control group. The authors suggested that the dose of VEGF determined whether the proliferation and activation of mesangial cells developed. As impaired capillary regeneration in severely damaged regions relates to the continuation of mesangial cell proliferation and activation, capillary regeneration can influence the suppression of mesangial cell proliferation and activation. In addition, recent studies suggested that VEGF does not affect the development of proteinuria in renal diseases[64, 65].

There are two studies suggestive of the relation between VEGF and several glomerular diseases. One study tested the urinary VEGF levels in patients with several glomerular diseases[66]. It appeared that normal subjects had detectable urinary VEGF excretion and, compared with them, the excretion was unchanged in minimal change disease (MCD), but was elevated in patients with FSGS and necrotizing glomerulonephritis. In contrast, MGN (membranous glomerulonephritis) was associated with the significantly suppressed urinary

excretion of VEGF, while decreasing clinical activity (reduction in proteinuria) was associated with recovery of urinary VEGF excretion toward the levels observed in the healthy subjects. The other study evaluated kidney tissue from 47 patients with a variety of renal diseases using immunohistochemistry and in situ hybridization[67]. The authors observed that VEGF expression was decreased or absent in sclerotic glomeruli, glomeruli compressed by crescents, glomeruli with a marked hypercellularity (SLE) and in areas occupied by matrix nodules (diabetic nephropathy).

Abnormal glycosylation of serum IgA has been shown in IgA nephropathy. A significant down-regulation of VEGF mRNA and protein in human mesangial cells was developed after incubation with aberrantly glycosylated IgA[68]. Depressed VEGF synthesis may play a role in the aberrant vascular repair and favor sclerosis in IgA nephropathy.

Collectively, VEGF expression seems to be associated with the nature and pattern of glomerular diseases, and less so with the disease itself. VEGF expression was decreased in the regions with sclerosis and impaired capillary density. One possible explanation is that VEGF expression is decreased in chronic renal lesions. Though it is not certain whether VEGF expression in glomerular diseases is playing a causative role or just results from the disease process, it appears that the beneficial effect of VEGF supplementation may have pathophysiological ground.

3. Diabetic Nephropathy

Several studies show that high glucose increases the production of VEGF in a variety of cell types[69-71]. The expression of VEGF mRNA and protein was enhanced after exposure to high glucose in both glomerular and tubular epithelial cells of the kidney[44, 72]. In addition, the up-regulation of VEFG and its receptors was demonstrated in the kidney of diabetic rats[73, 74]. In the study in OLETF rats described above, renal VEGF mRNA and glomerular VEGF immunoreactivity were reported to be increased over the duration of 9 to 68 weeks.

Besides the increased VEGF expression in hyperglycemia, there is evidence suggesting a potential pathophysiologic role of VEGF in DM nephropathy. High glucose induces VEGF gene and protein expression in several types of cells via protein kinase C, which is increasingly recognized as one of the central mediators of the damaging effects of hyperglycemia[72, 75, 76]. PKC inhibitor and PKC down-regulation inhibited glucose- induced increases in VEGF production[70]. In addition, a link between PKC and VEGF is further suggested by studies with an orally active PKCβ inhibitor[77, 78]. PKCβ inhibition was associated not only with reduced VEGF-induced retinal permeability but also with retardation of the development of albuminuria in streptozotocin-induced diabetic rats.

Another potentially important subject is the relation between VEGF and NO. VEGF exerts its angiogenic, vascular permeability and hemodynamic effects via activation and upregulation of eNOS in endothelial cells[79-81]. The action of NO as a downstream mediator of VEGF is commensurate with the up-regulation of the NO system in early diabetes and is implicated in the pathogenesis of early renal dysfunction. NOS blockade substantially reduces or even completely eliminates the hyperfiltration in experimental diabetes[82-84]. VEGF blockade prevented the up-regulation of eNOS in the diabetic glomeruli, thus supporting the contention that VEGF binds to its receptors on glomerular capillary endothelial cells and increases eNOS expression in these cells[85]. Along with this, interference with the VEGF-NO axis was shown to prevent microvascular dysfunction induced by high glucose level in experimental rats[86].

The expression of VEGF in the diabetic kidney may depend not only on the effects of hyperglycemia but on the effects of advanced glycated end products (AGEs) that accumulate in diabetic tissues over weeks and months[87]. These AGEs have been shown to activate VEGF expression in vivo and in vitro. Various other factors relevant to the pathogenesis of diabetic nephropathy have also been shown to promote VEGF expression, including stretch[88], angiotensin II[33], and a number of cytokines[27, 28]. Recently, one study showed that anti-VEGF antibody improved early renal dysfunction such as, hyperfiltration, albuminuria and glomerular hypertrophy in diabetic rats[85].

4. Renal Tumors

Since Folkman's proposal in 1971 that inhibition of angiogenesis may be a valid strategy for the treatment of solid tumors[89], extensive research has been dedicated to the identification and characterization of tumor angiogenesis factors. Many studies have demonstrated that VEGF is markedly up-regulated in the vast majority of human tumors so far examined, including lung, breast, gastrointestinal tract, bladder, ovary[90].

In the kidney, VEGF has been known as a major tumor angiogenic factor. Increased serum levels of VEGF in patients with renal cell carcinoma (RCC) have been reported and these levels were also increased in the affected kidney of patients with RCC[91]. Furthermore, VEGF expression in RCC correlated positively with the grade and size of the tumor[92, 93]. Therefore, VEGF expression is a potentially significant independent predictor of the outcome. Besides the RCC, angiogenesis in Wilm's tumor is also driven by VEGF[94].

5. Other Diseases

The VEGF mRNA and protein expression are enhanced in the experimental chronic cyclosporine (CsA) nephrotoxicity model[95]. CsA also increases the expression of VEGF receptor mRNA. Since chronic low-grade tissue hypoxia is believed to be important in the development of chronic CsA nephrotoxicity[96], CsA may cause injury to the endothelium, which may then up-regulate VEGF production as an adaptive mechanism directed toward repair and maintainance of the damaged endothelium. Actually, VEGF administration blunted the development of salt-sensitive hypertension and nephropathy after CsA exposure[97].

VEGF protein expression was shown to be up-regulated in the kidney of patients with chronic renal allograft rejection, a renal disease with histologic similarities to chronic CsA nephrotoxicity[98]. Inhibition of VEGF by topically applied neutralizing antibody markedly suppresses acute rejection of rat corneal allografts, indicating a contributory role for VEGF in the pathogenesis of rejection[99]. Recently, one study showed that VEGF gene polymorphism is associated with acute renal allograft rejection and may be a useful marker of the risk for rejection[100]. It is suggested that increased VEGF production enhances endothelial permeability and augments leukocyte infiltration of the allograft, which may promote a clinically recognizable rejection episode. However, the exact mechanism (s) and role of VEGF in the allograft rejection remain to be determined.

One recent study discovered increased angiogenesis and VEGF expression in the renal cysts in patients with adult polycystic kidney disease (ADPKD)[101]. The epithelial cells of some cysts and enlarged tubules in more preserved areas express VEGF, and some capillaries surrounding the cysts express VEGF R-2. It can be suggested that cyst development is associated with the local enhancement of the microvascular network which, in turn, may facilitate or promote fluid secretion into the cysts. The postulated molecular mechanism of this process involves VEGF secretion and stimulation of neovascularization.

Pharmacological tools to modulate VEGF in the kidney

1. Suppressing effects of VEGF

First of all, many attempts using the angiogenic effect of VEGF have been made at anti-tumor therapy. There is an extensive body of evidence documenting the fact that inhibition of VEGF activity results in suppression of the growth of a wide variety of tumor cell lines in murine models[102]. Furthermore, clinical trials in cancer patients are under way with several VEGF inhibitors, including a humanized monoclonal antibody and various small molecules, inhibiting VEGF signal transduction[102]. In the kidney, some reports show that anti-VEGF antibody alone or in combination with other anti-angiogenic drugs is effective in suppressing the growth of Wilm's tumor[102, 104]. Though not as well documented as the therapy of Wilm's tumor, there is a report that anti-angiogenic agents inhibit the expression of VEGF[105] in renal cell carcinoma. But, so far, there was no report about the direct relation between VEGF and renal cell carcinoma growth.

Based on the thought that an enhanced renal VEGF system is responsible for diabetes-associated renal changes, one report using anti-VEGF monoclonal neutralizing antibody in diabetic rats has recently been published[85]. Anti-VEGF antibody treatment decreased hyperfiltration, albuminuria and glomerular hypertrophy in streptozotocin-induced diabetes in rats. In an animal model of type II diabetes[106], it was shown that long-term AGEs-inhibitor treatment abolished the enhanced renal VEGF expression and afforded a renoprotective effect by reducing diabetes-induced renal collagen IV accumulation to normal levels and reducing the albumin excretion rate.

So far, the use of anti-VEGF agents in ADPKD and renal allograft rejection has not been explored. However, since it is possible that VEGF is associated with the pathogenesis of ADPKD and chronic renal allograft rejection[98, 101], anti-VEGF treatment has a potential for beneficial effects in those diseases.

2. Enhancing effects of VEGF

There have been numerous studies into the role of VEGF in renal failure. One study shows that VEGF blockade with an aptamer results in the inhibition of the capillary repair and leads to progressive renal damage[107]. In the model of experimental thrombotic microangiopathy, VEGF is capable of increasing peritubular capillary density, and this is associated with the amelioration of fibrosis and better preservation of renal function[56, 108]. Interestingly, while glomerular capillaries were repaired dramatically, peritubular capillary endothelium did not recover completely, and this was associated with a loss of VEGF from the tubular cells. VEGF can reduce the fibrosis and stabilize the renal function in the remnant kidney model[109]. In addition, in the CsA nephropathy, which is thought to be associated with chronic low-grade hypoxia, VEGF improves CsA-induced hypertension and nephropathy[97].

Masuda et al. demonstrated that angiogenic capillary repair plays an important role in recovery from severe glomerular damage in rats with experimentally induced glomerulonephritis[63]. Systemic administration of VEGF after acute glomerular injury successfully induced glomerular repair and

resolution of glomerulonephritis, associated with stimulation of angiogenesis and vascular remodeling. The use of VEGF as an angiogenic factor, therefore, could be potentially clinically useful for the treatment of glomerulonephritis accompanied by severe endothelial injury and capillary destruction.

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