CHAPTER II

LITERATURE REVIEW

Systemic Lupus Erythematosus (SLE) is a prototypic autoimmune diseases characterized by the production of autoantibodies that affects to multi system disorder. The causes of diseases are indistinct but believe genetic and environmental factors such as sunlight and drugs could trigger the disorder. There is a peak age of onset in young women between their late teens and early 40s and women to men ratio of 9:1. Ethnic groups, such as those with African or Asian ancestry, are at greatest risk of developing the disorder, which can be more severe than in white patients (1). Multi organs involvements in SLE are occur, especially vital organs, such as nervous system, heart, lungs, blood system and kidneys.

Lupus Nephritis

Renal disease is a common and serious manifestation of systemic lupus erythematosus (SLE). Lupus nephritis (LN) is one of the most severe complications in SLE, which involves kidneys including vascular, glomerular and tubulointerstitial lesions, and occurring in up to 60% of patients with SLE. It is one of the most leading causes of morbidity and mortality in systemic lupus erythematosus (SLE) (2). The presentations can range from asymptomatic urinary abnormalities to rapidly progressive renal failure leading to end-stage renal disease, the major issue among patients with lupus nephritis. The progression to LN in SLE is though to be dependent on the loss of self-tolerance and the formation of autoantibodies that deposit in the kidney to induce nephritis.

Etiology of Lupus Nephritis

Dysregulated apoptosis and inadequate removal of apoptotic cells and nuclear remnants may contribute to autoimmunity by causing prolonged exposure of the immune system to nuclear and cell membrane components (19). Recently studies have ascribed specific genetic linkage to the development of renal disease in SLE among certain ethnic groups, including European American and African American populations, some of which may determine the severity of the glomerular disease (2).

Pathogenesis and Mediation of Disease

The immune dysregulation in lupus nephritis is characterized by polyclonal B-cell activation, which induced by cognate autoreactive helper T-cells, and the formation of autoreactive antibodies directed against nuclear antigen and other self-antigen, so-call autoantigen (20). In general, lupus nephritis is associated with high titers of circulating high-affinity, IgG anti-double-stranded DNA (anti-dsDNA) antibodies and glomerular immunoglobulin deposits. Furthermore, elution of immunoglobulin from glomeruli revealed enrichment for anti-dsDNA antibodies. Therefore, it has been postulated that anti-dsDNA autoantibodies are nephritogenic in lupus nephritis. The binding of anti-double-stranded DNA (anti-dsDNA) autoantibodies to the glomerular basement membrane (GBM) in lupus nephritis can be explained by two mechanisms: (1) direct cross-reactive binding to intrinsic glomerular antigens; (2) nucleosome-mediated binding to heparin sulfate in the GBM (Figure 1) (21).

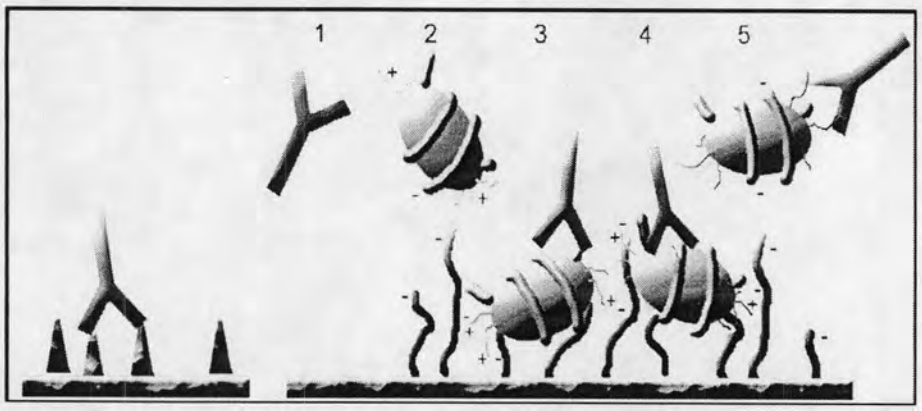

Figure 1. The two hypotheses for the glomerular binding of autoantibodies in lupus nephritis. From left to right: direct binding of cross-reactive autoantibodies to non-nucleosomal glomerular antigens; nucleosome-mediated binding of complexed autoantibodies to heparan sulfate (HS). The nucleosome-mediated binding does not occur with (1) non-complexed anti-nuclear antibodies or (2) free nucleosomes. Binding of (3) anti-dsDNA (blue) or (4) anti-nucleosome antibodies (green) will decrease the density of negative charges of the nucleosome. This will enhance binding of the complex to the negatively charged HS and lead to nucleosome-mediated binding. In contrast to this, binding of (5) anti-histone antibodies (red) to the nucleosome will decrease the amount of positive charges, which reduces the capacity to bind to HS in the glomerular basement membrane and preventing deposition of nucleosome/immune complexes (Kidney International 2007, 71: 600 601).

Renal Manifestations of Lupus Nephritis

The dominant feature of renal lupus is proteinuria (Table 1), present in almost every patient and commonly leading to the nephrotic syndrome. Microscopic hematuria is almost always present, but never in isolation; macroscopic hematuria is rare. Surprisingly, hypertension is not overall more common in those with nephritis than in those without; but, as expected, those with more severe nephritis are more commonly hypertensive. About half will show a reduced GFR, and occasional patients present with acute renal failure. Renal tubular function is disturbed, which is not surprising in view of

the finding of both immune aggregates in tubular basement membranes and the presence of interstitial nephritis (see below). In a high proportion of patients, urinary excretion of light chains and β2-microglobulin are both increased. Recently, hyperkalemic renal tubular acidosis has been emphasized as a manifestation of lupus (22).

Table 1. Clinical features of patients with lupus nephritis

Feature	% of Those with Nephritis
Proteinuria	100
Nephrotic syndrome	45 to 65
Granular casts	30
Red cell casts	10
Microscopic hematuria	80
Macroscopic hematuria	1 to 2
Reduced renal function	40 to 80
Rapidly declining renal function	30
Acute renal failure	1 to 2
Hypertension	15 to 50
Hyperkalemia	15
Tubular abnormalitiesb	60 to 80

Although, Some lupus nephritis patients with no clinical evidence of renal involvement (no proteinuria, normal urine microscopy, normal renal function) nevertheless showed active histological change on renal biopsy specimens. This has become known as "silent lupus nephritis" (4). Recently, the investigators found significant renal involvement (Class III, IV, or V LN) in SLE patients with < 1000 mg proteinuria with or without hematuria. These findings suggest that biopsy be strongly considered in this patient population (23).

The Classification of Lupus Nephritis

Based on various experimental models of autoimmune and immune complex disease in the kidney and observations in human renal biopsies, the classification of the various patterns of renal injury in SLE can be divided into three groups, including mesangial, endothelial and epithelial pattern (24).

The first World Health Organization (WHO) classification was formulated by Pirani and Pollak in Buffalo, New York in 1974 and was first used in publications in 1975 and 1978 (24). However, it is still doubtful for the value of the index and its reproducibility. In 1982, the WHO classification was modified by the International Study of Kidney Disease in Children. Diffuse proliferative lesion (type IV) is the most common and the most severe form of LN. Mesangial (type II) and membranous (type V) types are mild form and have relatively normal renal function. Focal proliferative type (type III) has quite variable clinical features. Progressive renal dysfunction is uncommon in type III with less than 25 percent of glomerular involvement (25).

Several studies have emphasized the usefulness of semiquantitative analysis to assess the activity and chronicity of nephritis (25, 26). The maximum of activity score is 24 and the maximum of chronicity score is 12. The score may be used as a prognostic index. However, it is still doubtful for the value of the index and its reproducibility. Recently, a newer classification modified from the WHO classification has been proposed. (Table 2) This new classification claimed the reproducibility and correlation to the long-term outcome (Table 3) (24). The incidence of class IV LN in Thai patients is higher than Caucasians (3)

Table 2. World Health Organization (WHO) morphologic classification of lupus nephritis (modified in 1982)

Class I	Normal glomeruli
	a. Nil (by all techniques)
	b. Normal by light microscopy, but deposits by electron or immunofluorescence microscopy
Class II	Pure mesangial alterations (mesangiopathy)
	a. Mesangial widening and/or mild hypercellularity
	b. Moderate hypercellularity
Class III	Focal segmental glomerulonephritis (associated with mild or moderate mesangial alterations)
	a. With "active" necrotizing lesions
	b. With "active" and sclerosing lesions
	c. With sclerosing lesions
Class IV	Diffuse glomerulonephritis (severe mesangial, endocapillary or mesangiocapillary proliferation and/or extensive subendothelial deposits)
	a. Without segmental lesions
	b. With "active" necrotizing lesions
	c. With "active" and sclerosing lesions
	d. With sclerosing lesions
Class V	Diffuse membranous glomerulonephritis
	a. Pure membranous glomerulonephritis
	b. Associated with lesions of class II
	c. Associated with lesions of class III
	d. Associated with lesions of class IV
Class VI	Advanced sclerosing glomerulonephritis

Table 3. International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification of lupus nephritis.

Class I	**Minimal mesangial lupus nephritis**
	Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	**Mesangial proliferative lupus nephritis**
	Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits
	May be a few isolated subepithelial or subendothelial deposits visible by immunofluorescence or electron microscopy, but not by light microscopy
Class III	**Focal lupus nephritis[a]**
	Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations
Class III (A)	Active lesions: focal proliferative lupus nephritis
Class III (A/C)	Active and chronic lesions: focal proliferative and sclerosing lupus nephritis
Class III (C)	Chronic inactive lesions with glomerular scars: focal sclerosing lupus nephritis
Class IV	**Diffuse lupus nephritis[b]**
	Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental(IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation
Class IV-S (A)	Active lesions: diffuse segmental proliferative lupus nephritis
Class IV-G (A)	Active lesions: diffuse global proliferative lupus nephritis
Class IV-S (A/C)	Active and chronic lesions: diffuse segmental proliferative and sclerosing lupus nephritis
	Active and chronic lesions: diffuse global proliferative and sclerosing lupus nephritis
Class IV-S (C)	Chronic inactive lesions with scars: diffuse segmental sclerosing lupus nephritis
Class IV-G (C)	Chronic inactive lesions with scars: diffuse global sclerosing lupus nephritis
Class V	**Membranous lupus nephritis**
	Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations
	Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed
	Class V lupus nephritis show advanced sclerosis
Class VI	**Advanced sclerosis lupus nephritis**
	≥90% of glomeruli globally sclerosed without residual activity

[a] Indicate the proportion of glomeruli with active and with sclerotic lesions.
[b] Indicate the proportion of glomeruli with fibrinoid necrosis and or cellular crescents.
Indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, severity of arteriosclerosis or other vascular lesions.

Vascular Endothelial Growth Factor (VEGF)

The vascular endothelial growth factor gene family consists of VEGF-A (here after referred to as VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E (viral homologous of VEGF), placental growth factor (PLGF) and VEGF-F (snake venom-derived VEGFs) **(27)**. These glycoproteins belong to a structural superfamily of growth factors which includes PDGF. All of them have a common structure of eight cysteine residues in a VEGF homology domain **(28, 29)**. Table 4 shown as VEGF gene family and it property **(27, 30, 31)**. The action of VEGF and other family members is mediated by a particular family of receptor tyrosine kinases (RTKs), VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGR-3 (Flt-4), which are expressed almose exclusively on endothelial cells. In addition, neuropilin-1 and neuropilin-2 a transmembrane protein involed in regulation of axonal guidance in neurons, has been described as a corecepter for VEGF **(28, 32)** (Figure 2). The members of VEGF family have different physical and biological properties. The VEGFR-3 receptor and its ligands, VEGF-C and VEGF-D, are associated with lymphangiogenesis, while PLGF is linked to arteriogenesis **(33)**. Each VEGF ligands have difference biological activities of VEGF (Table 5) **(34)**.

Table 4. The chromosomal localization, major mRNA transcript and major sizes of the VEGF ligands

Gene	Sequence homology	Chromosomal localization	Splice variants	Major mRNA transcript size (kb)	Major protein size (kDa)
VEGF		6p21.1	121, 145, 165, 183,189, 206	3.7, 4.5	21
VEGF-B	45% homology with VEGF-A	11q13	167, 186	1.4	21, 30
VEGF-C	30% homology with VEGF-A_{165}	4q34	-	2.4	20-21
VEGF-D	61% homology with VEGF-C; 31% with VEGF-A_{165}	Xp22.31	-	2.2	20-21
VEGF-E	20-25% homology with VEGF-A	viral homologous	N/A	N/A	N/A
PLGF	42% homology with VEGF-A	14q24	131, 152, 219	1.7, 1.2	38,30
VEGF-F	50 % homology with VEGF-A_{165}	Snake venom	N/A	N/A	N/A

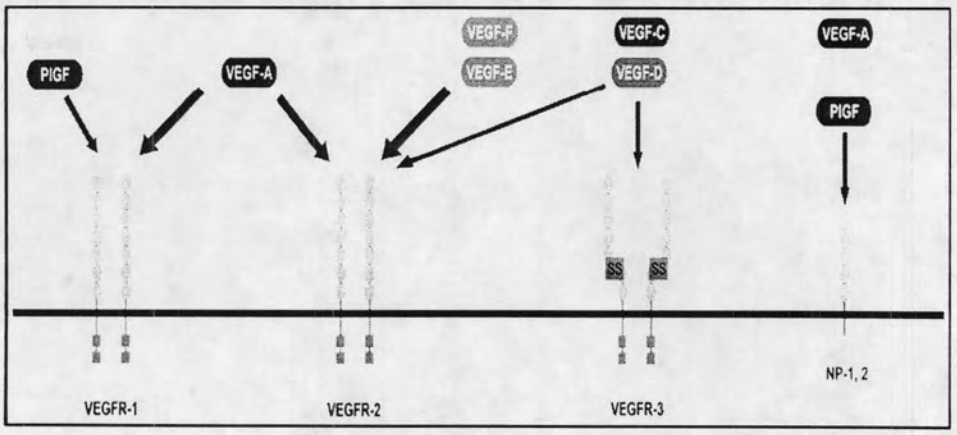

Figure 2. Interaction of VEGF family members with the VEGFR-1, -2 and -3, and neuropilins 1 and 2. (Zaher K. et al., Blood Cells, Molecules, and Diseases, 2007)

Table 5. The biological activities of VEGF ligands (34).

	EC proliferation	EC migration	Vascular permeability	Angiogenesis	Lymphangiogenesis
VEGF-A	+	+	+	+	+, -[1]
VEGF-B	-	ND	-	+, -[1]	-
PIGF	-	+	-	+[2]	ND
VEGF-C	+	+	+	+[2]	+
VEGF-D	+	+	–	+[3]	+
VEGF-E	+	+	+	+	ND
VEGF-F1[4]	+	ND	+	+	ND
VEGF-F2[5]	+	+	+	+	ND

ND: not Determined, (1) Conflicting reports (see Text), (2) Modest angiogenic activity, (3) Full-length form of VEGF-D induces modest angiogenesis, while mature form of VEGF-D (VEGF-DΔNΔC) strongly induces

The VEGF Structure and Function

The human gene for VEGF (also referred to as VEGF-A) resides on short arm of chromosome 6 (6p21.3). The coding region spans ~14 kb and contains eight exons. Alternative splicing of a single pre-mRNA generates several distinct VEGF species. At least six VEGF isoforms of variable amino acid number are produced through alternative splicing **(35)**. VEGF was firstly described by Senger et al. in 1983 **(36)**, as a homodimeric 34 42 kDa protein that increased vascular leakage in the skin. This protein was called tumor vascular permeability factor (VPF) and was isolated from ascetic fluid and cell culture supernatants of a guinea-pig hepatocarcinoma cell line. In 1989, Ferrara and Henzel **(37)** identified an endothelial growth substance that received the name of VEGF. In subsequent works it was established that VEGF and VPF were the same molecule **(29, 38, 39)**.

VEGF has endothelial cell-specific mitogenic activity and stimulates angiogenesis in vivo. This specificity of mitogenicity separates VEGF from the majority of other growth factors which stimulate proliferation in several cell types. VEGF also stimulates vascular permeability, with an effect 50,000 times greater than that of the vasoactive substance histamine **(29, 31)**. Numerous studies addressing the expression and function of VEGF emphasize the importance of this factor in physiological and pathological angiogenesis, and vascular permeability. In addition, it also promotes monocyte chemotaxis and expression of adhesion molecules **(40)**.

VEGF has significantly homology to PDGF, and all the eight cysteines found in the A and B chains of PDGF are conserved in VEGF. VEGF is a heparin-binding homodimeric glycoprotein of 45 kDa. This property closely corresponds to those of VEGF165, which is indeed the major VEGF isoform. To date, the human VEGF gene is composed differentially spliced to yield four mature isoforms (VEGF121, VEGF165, VEGF189 and VEGF206) (Figure 3). The numeric designation of the isoforms denotes the number of amino acids in the molecule. In addition, some less commonly expressed isoforms were identified (VEGF145 and VEGF183) **(27, 29, 31, 33, 40)**. Through alternative mRNA splicing, the VEGF isoforms differ by the presence or absence of

sequences encoded by exons 6 and 7. VEGF165, VEGF189, and VEGF121 differ in affinity for heparin and heparan-sulfate proteoglycans (VEGF189 > VEGF165 > VEGF121) and in mitogenic effect (VEGF165 > VEGF121). VEGF165, VEGF189, and VEGF206 are in most part sequestered in the extracellular matrix (ECM) and the cell surface, whereas VEGF121 and VEGF145 are freely released **(40)**. VEGF isoforms in the ECM constitute a reservoir of growth factor that can be slowly released by exposure to heparin, heparan sulphate and heparinases or more rapidly mobilised by specific proteolytic enzymes such as plasmin and urokinasetype plasminogen activator (uPA). These enzymes already contribute to angiogenesis through ECM depolymerisation and, as well as releasing sequestered VEGF from the cell surface and ECM, might also regulate VEGF bioactivity **(35)**.

VEGF exerts its biologic effect through interaction with cell-surface receptors. These receptors are transmembrane tyrosine kinase receptors and they include VEGF receptor-1 (VEGFR-1; Flt-1) and VEGFR-2 (kinase insert domaincontaining receptor/Flk-1), selectively expressed on vascular endothelial cells, and the neuropilin receptors (NP-1 and NP-2), expressed on vascular endothelium and neurons. Upon binding of VEGF to the extracellular domain of the receptor, a cascade of downstream proteins is activated after the dimerization and autophosphorylation of the intracellular receptor tyrosine kinases. VEGFR-2 appears to be the main receptor responsible for mediating the proangiogenic effects of VEGF **(27)**.

VEGF is the most potent pro-angiogenic protein described to date. It induces proliferation, sprouting and tube formation of endothelial cells (ECs) **(32)**. It is also a potent survival factor for ECs and has been shown to induce the expression of anti-apoptotic proteins in these cells **(41)**. VEGF also causes vasodilatation by inducing the endothelial nitric oxide synthase and so increasing nitric oxide production **(42)**. VEGFR binds many receptors on hematopoietic stem cells (HSCs), monocytes, osteoblasts and neurons. It induces HSC mobilization from the bone marrow, monocyte chemoattraction and osteoblast-mediated bone formation **(32)**. Hypoxia is the main stimulus for VEGF expression and/or production. Several growth factors and cytokines

such as epidermal growth factor, transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor I (IGF-I), angiotensin II, interleukin-1 (IL-1), and IL-6 also have the potential to up-regulate VEGF expression. VEGF may be induced by other factors as well [i.e., prostanglandins, mechanical stress, hyperglycemia, advanced glycation end products (AGEs), protein kinase C (PKC), and reactive oxygen species (ROS)]. Angiopoietins and VEGF play co-ordinate and complementary roles in vascular homeostasis (8).

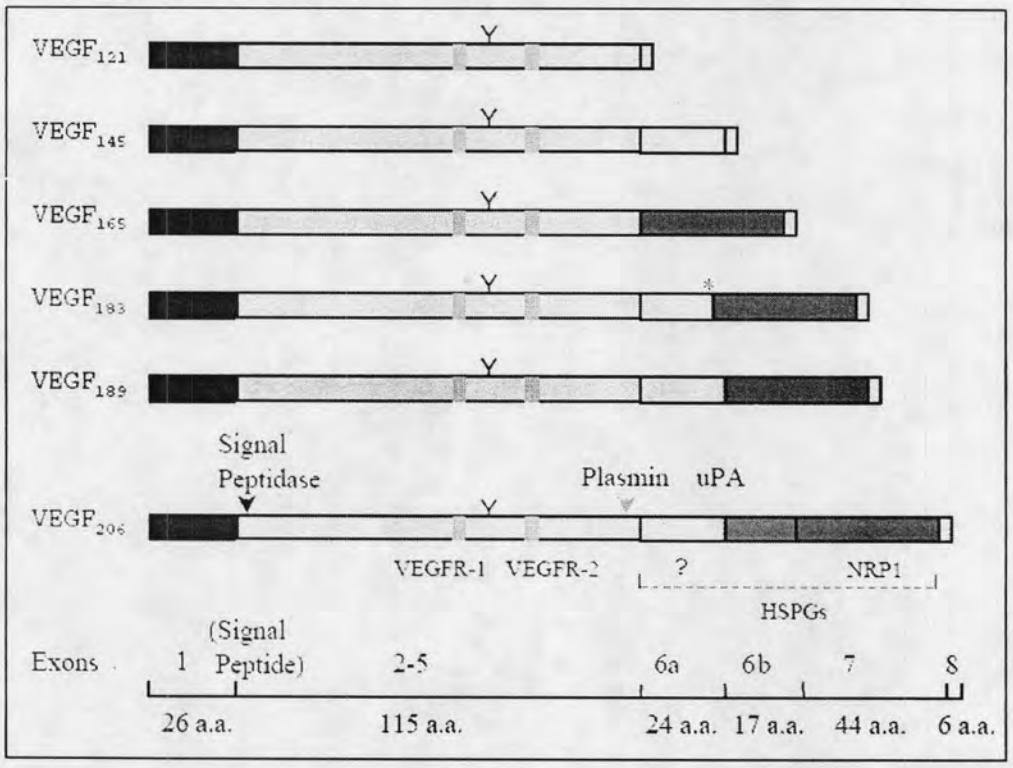

Figure 3. The splice variants of human VEGF. The human VEGF gene, through alternative mRNA splicing, produces six isoforms, which differ by the presence or absence of sequences encoded by exons 6 and 7. Domain sizes (a.a., amino acid residues) and the exons from which they derive are shown at the foot of the figure. Sites of interaction with VEGFRs, NRP1 and HSPGs are indicated on the VEGF206 isoform, as are certain enzyme cleavage sites. Note that it is not known where uPA cleaves the exon-6-encoded region in relation to the truncation point of this domain within VEGF183 (*). The potential N-glycosylation site is indicated by 'Y', whereas '?' represents binding to unknown components at the cell surface and in the ECM.

In vivo, VEGF expression has been shown to be associated with significant steps in angiogenesis and physiologic vasculogenesis (43). In mice, deletion of the VEGF gene is lethal, resulting in vascular defects and cardiovascular abnormalities (44). VEGF affects an important number of angiogenic processes including wound healing, ovulation, maintenance of blood pressure, menstruation and pregnancy (45). Transgenic mice, over-expressing VEGF in the skin, have abundant cutaneous angiogenesis and a psoriasis-like skin condition (46). Over-expression of VEGF in mouse skin also accelerates experimental tumor growth (47). In contrast, mice with a mutated VEGF exhibit delayed wound healing (48). In humans, VEGF is expressed in practically all solid tumors studied as well as in some hematological malignancies (32). Recently, VEGF (specifically VEGF189) was overexpressed in patients with distant metastases of pulmonary adenocarcinoma (49). In fact, correlations have been found between the level of VEGF expression, disease progression and survival of several cancers (47, 50). It was shown that VEGF165, the predominant isoform, may be a significant biological indicator of the invasiveness of postoperative recurrence of hepatocellular carcinoma (51). VEGF189 is thought to be most potent for vascularization in various cancers (52).

VEGF in Renal Physiology

The maintenance of normal vasculature is certainly critical for organ function, especially for the most highly vascularized organ, the kidney. The role of VEGF in normal renal physiology is essentially unknown. The maintenance of glomerular capillary number is critical for glomerular filtration whilst peritubular capillaries (PTCs) are essential for providing oxygen and nutrients to the tubules and interstitial cells. In addition, within the developing glomerulus, all of the major isoforms of VEGF are highly expressed in presumptive and mature podocytes, although the 164 isoform predominates (53). In the kidney, VEGF is primarily localized to the glomerular podocyte and to tubular cells, especially in the outer medulla and medullary rays, which are normally at a borderline hypoxic status (54). The expression of the different VEGF

isoforms in normal human glomeruli was complex and variable with substantial inter- and intra-individual variation. The receptors for VEGF ligands, including VEGFR-1, VEGFR-2 and neuropilin-1 are expressed by the adjacent endothelial cells, and neuropilin-1 has also been identified in podocytes (55). In contrast, *In vitro* study indicated that VEGF121, VEGF165 and VEGF189 mRNA/protein expressed by rat and human mesangial cells (56) also as VEGFR-1 and VEGFR-2 (8). For VEGF expression control, angiopoietin-1 (Ang-1), but not Ang-2, was identified in adult human glomeruli, particularly in podocytes (57). Ang-1 is known to antagonize some of the effects of VEGF-A, including the induction of permeability in endothelial cells, and it is also believed to help stabilize newly formed vessels (58). Tie-2 was demonstrated in glomerular capillary endothelial cells of human and rat glomeruli and in cultured human microvascular endothelial cells (59).

VEGF in Renal Pathophysiology

Given the role of VEGF in promoting microvascular permeability, it had been speculated that VEGF may regulate glomerular permeability, although it is generally acknowledged that the capillary fenestrations do not represent the ultimate barrier to filtration. In contrast to the prominent expression of the VEGF system in the adult kidney, the administration or inhibition of VEGF in normal adult appears to have only minimal effects. In the isolated perfused rat kidney, administration of VEGF increased the renal blood flow but did not influence the glomerular filtration rate or the permelectivity of the glomerular barrier wall (60). The administration of neutralizing monoclonal anti-VEGF antibodies to normal rats had no effect on glomerular filtration rate or glomerular volume (61). In contrast, the evaluation of anti-VEGF neutralizing antibodies and sFlt-1 in the mice caused to proteinuria and rapid glomerular endothelial cell detachment and hypertrophy (62). The podocyte-specific heterozygous and homozygous deletions of VEGF in mice resulted in proteinuria and endotheliosis by 2.5 weeks of age, and in perinatal lethality, respectively, with loss of endothelial feneatrations or failure to form fenestrations while it overexpression of VEGF165 led to a collapsing glomerulopathy (12).

Recently, selective knockout of VEGF in the podocyte has been achived using a Cre-recombinase nephrin promoter and Lox-VEGF mice, which showed impaired glomerular capillary formation due to a loss of endothelial cells, suggesting an important role for podocyte VEGF in maintaining capillary integrity (12). Kang *et al.* found a remarkable loss of VEGF in both the podocytes and in the tubules in the outer medulla, and the loss of tubular VEGF expression correlated with the severity of peritubular capillaries loss (PTCs) in aging-associated renal disease rat (63) and remnant kidney (RK) rat models (13). Moreover, in RK model, VEGF treatment resulted in reduces renal fibrosis and improved renal function (14).

VEGF plays a crucial molecule in the progression of many renal diseases such as diabetic nephropathy, thrombotic thrombocytopenic purpura and glomerulonephritis (64, 65). Both animals and patients with type 1 and type 2 diabetes had renal VEGF and its receptors expression, especially early in the course of diabetes. Inhibition of VEGF resulted in benefical effects on diabetes-associated renal changes, underlining a deleterious role for VEGF in the pathophysiology of diabetic nephropathy. The cause of the up-regulation of the VEGF system in diabeties remains unknown (8).

Vascular Endothelial Growth Factor (VEGF) in Lupus Nephritis

In systemic lupus erythematosus, high levels of VEGF have been correlated to lupus activity and specifically to nephritis. The VEGF levels were higher in systemic lupus erythematosus (SLE) patients with renal dysfunction compared to SLE without renal damage, primary antiphospholipid syndrome, and normal controls. In addition, overexpression of VEGF in epithelial cells and podocyte on renal tissue was demonstrated (16).

In a recent study that evaluated the levels of VEGF and its receptors in SLE patients, the author could demonstrate that VEGF and the soluble VEGFR-1 concentrations were higher in patients with active lupus than in inactive disease or healthy people. On the other hand, soluble VEGFR-2 was lower in this group of active

lupus patients than in inactive group. They concluded that the imbalance between VEGF and its soluble receptors might have a role in SLE pathogenesis (17). *In vivo* study, NZBxNZW mice that received anti-VEGFR-2 had a higher mortality and an accelerated renal disease compared to controls. Anti-VEGFR-2 might exacerbate renal disease by disrupting glomerular endothelial functioning or facilitating immune complex deposition (66).

Nishitani et al. (67) measured VEGF mRNA in peripheral blood mononuclear cells of 34 patients with lupus nephritis, but they did not find any difference between lupus and healthy populations. The measurement of urinary VEGF mRNA by quantitative real-time PCR could predict class IV of lupus nephritis from others classes in patients with high levels of this substance. Additionally, a significant reduction of VEGF mRNA levels was observed in patients who responded to therapy. It brings a possible additional noninvasive tool for monitoring lupus nephritis (5). In the same way, the studies in urinary of renal disease presented the variable glomerular podocytes associate with the glomerular sclerosis and loss of renal function (68). Moreover, the urinary podocytes were high number in lupus nephritis patients who had active stage and its disappeared after treatment (69).