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Kidney vasculogenesis and angiogenesis: role of Vascular Endothelial Growth Factor

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Abstract. – **Vascular Endothelial Growth Factor (VEGF) plays a crucial role in the estabilishment of the vascular tree pattern.**

New vessels can be formed by two different ways; in the development of kidney both vasculogenesis and angiogenesis partecipate to microvessel assembly. VEGF and its receptor (VEGF-R) are co-expressed during kidney organogenesis and stimulate renal blood vessels development, induce and mantain the fenestred phenotype in endothelium and regulate vascular permeability.

VEGF and many other growth factors partecipate to the development of embrionic glomerular microvasculature.

We believe that therapeutical use of VEGF or anti-VEGF antibodies may be performed in the treatment of many disorders.

Key Words:

Vascular Endothelial Growth Factor, Kidney, Angiogenesis, Vasculogenesis.

Introduction

After the implantation vascular and hematopoietic tissues develop together[1].

In the absence of any pre-existing vessels, mesodermally-derived cells differentiate into either endothelial progenitor cells (angioblasts) or into primitive hematopoietic lineages. Differentiation of precursor cells into endothelia and assembly of endothelial progenitor into vessels occur during vasculogenesis.

In contrast, during angiogenesis pre-existing vessels branch, sprout and migrate to form new capillaries[2].

New vessels can be formed by two different ways: vasculogenesis and angiogenesis. Early in gestation first endothelial cells are formed by vasculogenesis; later in gestation probably both vasculogenesis and angiogenesis participate to vascular growth[3].

Many citokines partecipate to the development of vascular system, of them Vascular Endothelial Growth Factor (VEGF) play a crucial role in the estabilishment of the vascular tree pattern[4] (Figure 1).

VEGF and vascular tree development

VEGF, a dimeric glycoprotein, member of the platelet derived growth factor family[5], is an important regulator of endothelial cell (EC) proliferation and migration and plays a crucial role in regulation of microvessels permeability and in inducing vasodilation[4-6].

VEGF m-RNA is expressed by a wide variety of non endothelial cells in location where endothelia are proliferating: smooth muscle cells, fibroblasts, epithelial cells (podocytes in kidney); macrophages and tumor cells may produce VEGF, indicating, for this cytokine, a role in the angiogenesis of wound healing and tumour growth[7].

Hypoxia and some peptide growth factors induce VEGF expression: fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor beta (TGF-b), platelet-derived growth factor (PDGF), placenta growth factor (PlGF), interleukin 6 (IL-6) and interleukin 8 (IL-8)[8].

bFGF is the most potent angiogenic factor known. Recent reports have demonstated that vascular development requires interaction of VEGF and bFGF, indeed both partecipate to differentiation of mesenchymal cells into endothelial cells[9].

Many evidences lead to the hypotesis that TGF-β plays a role in mediating cell-extracellular matrix interaction and that also extracellular matrix may regulate the formation of

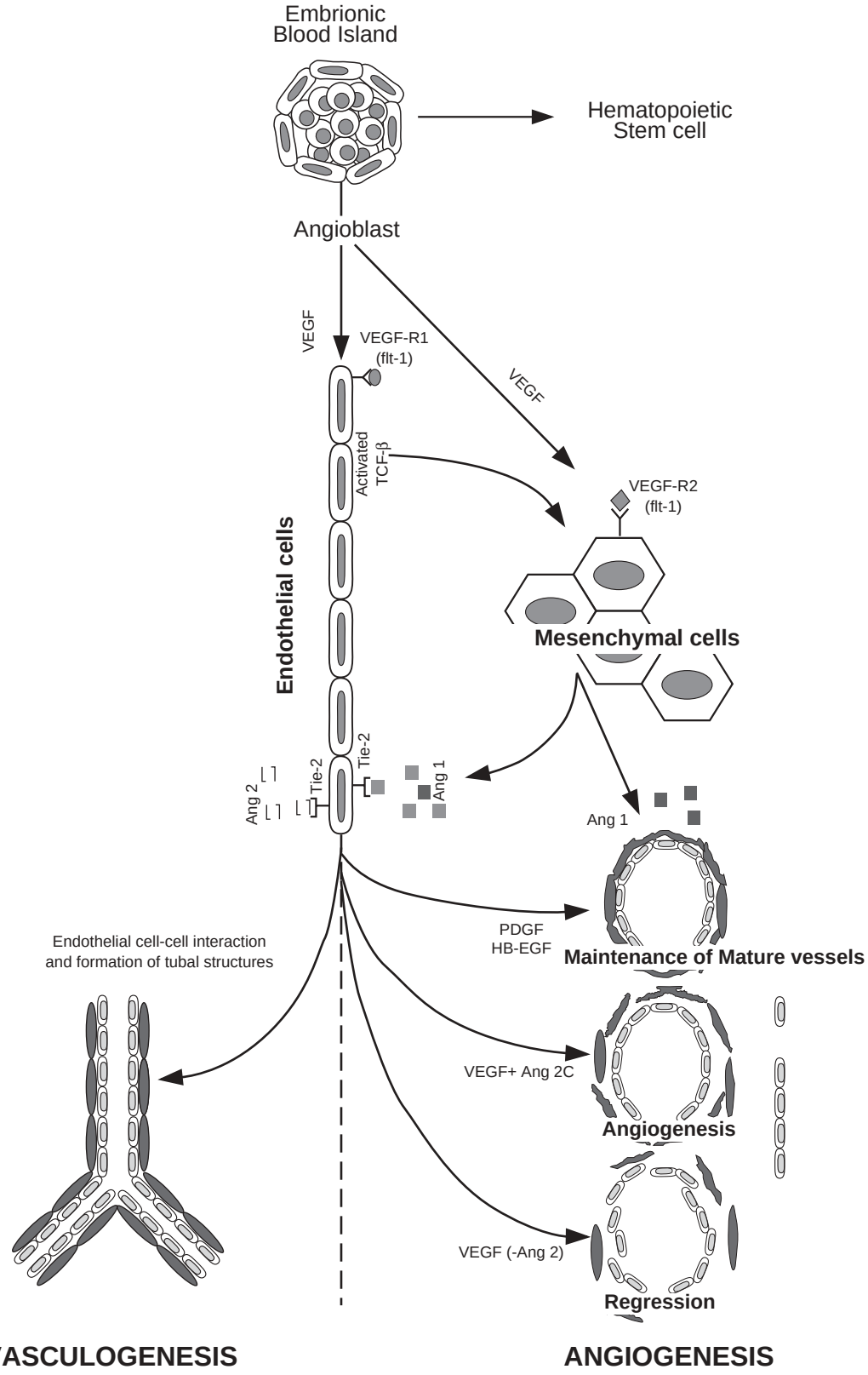

Figure 1. The vascular tree development: coordinate and subsequent actions of VEGF and other cytokines in vasculogenesis and angiogenesis. Role in the manteinance of mature vessels by reclutation of mesenchymal cells, inihibition of EC proliferation and accumulation of extracellular matrix. Role in vascular regression by loss of structure and matrix contact and decrease of growth signals.

capillary-like tubles. Moreover lower concentration of TGF-β potentiates the effect of bFGF and VEGF[10].

There are two different tirosine kinase receptors for VEGF (VEGF-R): Flt-1 or VEGF receptor 1 and Flk-1 or VEGF receptor 2.

Studies on mice demonstrate that Flk-1 is the first marker of endothelial progenitors; during embrionic development VEGF may support differentiation of flk-1 positive cells into endothelial cells. Flt-1 endothelial cells assemblate in vascular channels; VEGF supports cell-cell and cell-matrix interactions and thus provide to embrionic vasculature organization. Flk-1 may interact with other ligands other than VEGF and all play a role in early steps of endothelial differentiation[11], flt-1 expression on endothelial cells occurs later in embrionic development and its interaction with VEGF permits vessels organization[12].

Some authors have reported another tirosine-kinase receptor for VEGF: VEGF-R3 that is later expressed only on the surface of the ECs that will become vein or lymphatic vessels[3].

Many studies demonstrate the importance of the interaction VEGF/VEGF-R for endothelial differentiation in the mouse embryo: flk-1 deficient mice die in utero because of an early defect in the development of hematopoietic cells and endothelial cells. Flt-1 null mutant mice die later in utero. In these endothelial differentiation is not blocked, but there is a disorganization of the early embrionic vasculature[13].

VEGF levels are high during embriogenesis and fetal development and in adulthood only in hypervascularised tisses such as ovarian corpus lutheum and in proliferating endometrium during the menstrual cycle[14].The continous expression of both VEGF in epithelial cells, such as podocytes and of VEGF-R on glomerular endothelium, induces and manteins the fenestred phenotype in endothelium,and also regulates vascular permeability[15].

VEGF and kidney

Kidney is formed by an intricate system of microvascular units. Each nephron microvascular unit is formed by an afferent glomerular arteriole, a glomerular capillary tuft, an efferent glomerular arteriole and a peritubular capillary bed[16]. Vasculogenesis and angiogenesis probably partecipate to microvessel assembly in the development of kidney[17].

VEGF and VEGF-R are co-expressed during kidney organogenesis and stimulate renal blood vessels development: epithelia of ureteric bud and the avascular renal mesenchyme express VEGF mRNA throughout nephrogenesis; podocytes and collecting duct epithelial cells express VEGF mRNA later in nephrogenesis[18].

At the onset of nephrogenesis, flk-1 is expressed by undifferentiated renal mesenchymal cells and provides to vasculogenesis, later both flk-1 and flt-1 are expressed by endothelial cells in glomeruli and around tubules to support angiogenesis[19].

The epithelial cells adiacent to fenestred endothelium in the glomerulus show an high constitutive expression of VEGF; the endothelial cells express VEGF-R. Continous expression of VEGF in epithelial cell podocytes and expression of VEGF-R in glomerular endothelium, induce and mantain the fenestrated fenotype in endothelium[15]. The presence of fenestred endothelium in kidney permits filtration, secretion, and absorbition[20].

Although high levels of VEGF are found during embrionic development in the brain and kidney, VEGF levels decrease only in adult brain; glomerular podocytes continue to express VEGF, at the same manner VEGF-R levels are high in the brain and kidney during embriogenesis, but only glomerular endothelium express VEGF-R into adulthood. All these evidence confirme the hypotesis that VEGF levels are related to vessels permeability: high premebility of fenestred glomerular endothelium in adults is related to high levels of VEGF, in contrast low permeability of blood brain barrier in adult is related to low VEGF levels[20].

Hypoxia seems to play a crucial role in stimulating VEGF production by glomerular epithelial mass and VEGF-R expression in endothelial precursor cells. Some studies demonstrate the induction of VEGF and VEGF-R expression in culture of metanephric kidney under hypoxic conditions and indicate a role for VEGF mediation of vasculogenesis by recruitment of flk-1 expressing angioblasts[21].

Hypoxia is a rapid and potent inducer of VEGF mRNA expression. Hypoxia Inducer

Factor (HIF) binds the VEGF enhancer and thus induces VEGF mRNA trascription. HIF-1 is a helix-loop-helix (HLH) PAS family of trascriptor factors that regulate the trascription of many hypoxia inducible factors, such as erythropoietin. Distruction of HIF binding site inhibits hypoxia inducibility of VEGF[22].

Hypoxia inducing factor Related Factor (HRF), another HLH Pas family member, is expressed by endothelial cells of brain capillary, kidney glomeruli and chorioid plexus in the embryo and adult. Moreover podocytes express HRF that involve a role in the regulation of vascular permeability by manteinance of VEGF/VEGF-R high levels[23]

Several growth factors and their receptors partecipate to the development of embrionic glomerular microvasculature through cooperative effects: VEGF, FGF, PlGF, TGF-β, PDGF, HGF (Hepatocyte growth factor) and angiopoietin 1 family[24].

The vascular branching and vessel remodelling is regulated by angiopoietin 1 and 2. Specific tirosine kinase receptors for angiopoietin family are tie1 and tie2. Tie2 may bind both angiopoietin 1 and angiopoietin 2: angiopoietin 1 stimulates the differentiation of surrounding mesenchym into pericyte or smooth muscle. Angiopoietin 2 levels increase following angiopoietin 1 expression and only at sites of active vessel remodelling. The interaction of angiopoietin 2 with tie2 inhibits receptors activation[24-25].

Tie2 and its ligands angiopoietin 1 and 2 interaction seems to accelerate more than inhibit the vascular development. This observation suggests a mayor role of angiopoietin 1 in vessels remodelling[25].

PDGF and angiopoietins also reclutate perycites cells to their endothelial partners.

Another ligand-receptor system has been hypotised to mediate cell-cell recognition: ephrin A, ephrin B and their receptors Eph A and B play a role during embrionic development in definig the vessels organizational plan.

Interaction between ephrins and their receptors causes cell aggregation and promotes organizational responses. EphA2 receptors family partecipate to angiogenesis and chemotactic responses. EphB1 receptors family is most expressed in human renal microvascular endothelial cells and in glomerular endothelial cells. Some studies indicate that ephrin B1 and its receptor EphB1 are expressed in developing and mature murine glomeruli. In the adult mouse kidney, mesenchymal and interstitial cells do not express ephrinB1 and its receptor, while arteriolar intimal cells and glomerular capillary endothelial cells express them. EphrinB1/EphB1 system induces cultured human renal microvascular endothelial cells to assemblate into capillary like structures. These observations lead to the hypotesis that ephrin/Eph interaction partecipates in the cell-cell recognition processes required for glomerular capillary assembly[26].

Conclusions

Neoangiogenesis or its inhibition are the most mechanism leading to many pathologies.

VEGF levels increase during neoangiogenesis related to many diseases such as wound healing, tumour, proliferative retinopathy and cutaneous disease[7].

In glomerular injury models, repair is mediated by angiogenesis and it has been hypotised that angiogenesis recapitulates developmental models[27].

Neutralizing antibodies against VEGF and bFGF reduce the endothelial proliferation, this observation leads to the hypotesis that local production of VEGF and bFGF are increased where ECs proliferate to repair glomerular damage[24].

Use of monoclonal antibodies in therapy has been ipotised to block neoangiogenesis.

Some studies demonstrate that anti VEGF antibodies inhibit tumoral growth, riduce number and size of metastases; we can thrust this observation to each other hypervascularised disease, such as proliferative retinopathies and rheumtoid arthritis[28]. In contast intramuscolar or intra-arterial administration of VEGF augments perfusion and collateral vessels development as it has been evidencied in a rabbit model of chronic hindlimb ischemia[29]. We ipotise that this cytokine may be used in organs transplantation or in other pathologies characterized by an impair in tree vascular development such as congenital kidney malformation.

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