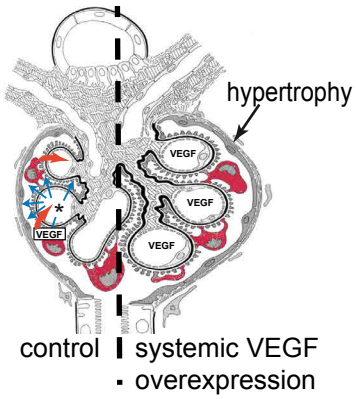
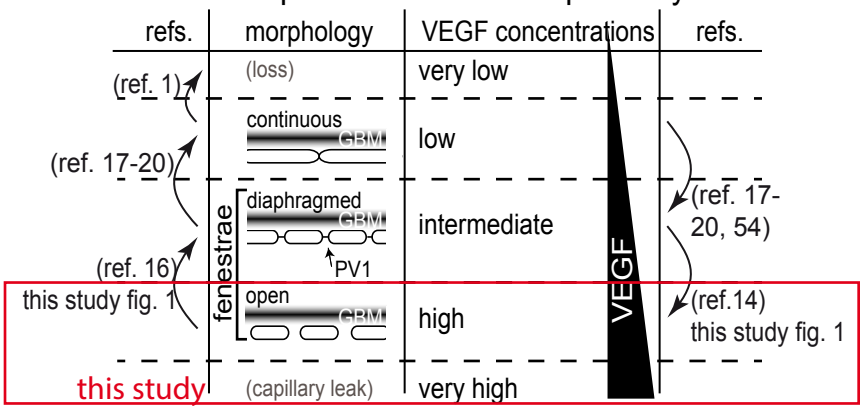
Supplemental Figures and Legends

supplemental Figure S1

A Glomerular VEGF action



B VEGF-dependent endothelial plasticity

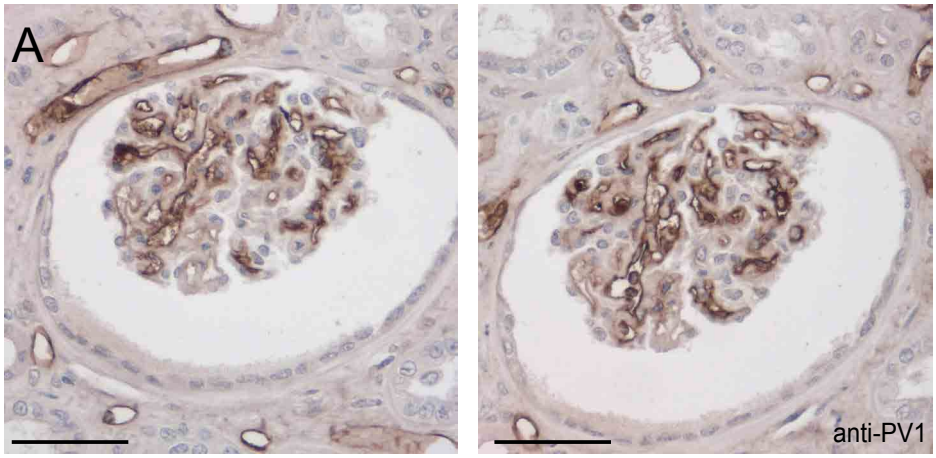


Supplemental Figure S1

A. Schematic of VEGF actions in the renal glomerulus. Left side: **Under physiological conditions**, VEGF is secreted from podocytes (red) into the primary urine. From theoretical considerations [1], about 30% of the secreted VEGF are estimated to be back-filtered by diffusion (orange arrows) across the glomerular filtration barrier against the flow of the filtrate (blue arrows) to endothelial cells. Right side: When VEGF levels are artificially increased within the blood or in podocytes, glomeruli undergo hypertrophy [2]. **B. Model of endothelial plasticity to detect VEGF levels *in vivo.*** Increasing VEGF action induces a specific sequence of morphological changes in endothelial cells: Continuous endothelial cells without pores > diaphragmed fenestrae (PV-1 positive) > open fenestrae (PV-1 negative). Experimental validations of endothelial changes in response to changes in VEGF activity are indicated (for references see reference list in the main body of the manuscript, ref. 54; Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J Cell Sci 1995;108 (6):2369-2379).

supplemental Figure S2

serial sections of an incidental atubular glomerulus



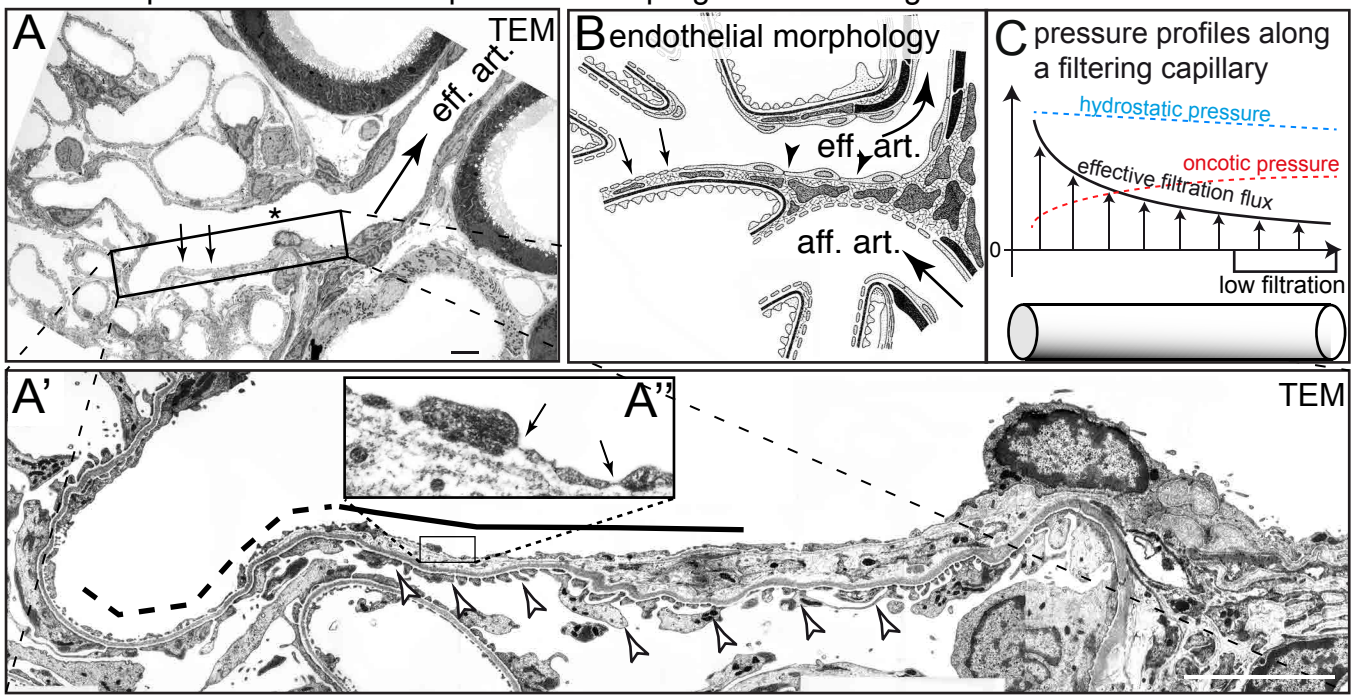
Supplemental Figure S2

Serial immunostainings for PV-1 in human biopsies

A. Anti-PV-1 staining revealed ubiquitous expression of PV-1 in incidental atubular glomeruli (as defined by serial sections, a smaller glomerular tuft and absence of tubular orifice). (Scale bars 50 µm)

supplemental Figure S3

Filtration pressure reduction/equilibrium and progressive sealing of the efferent arteriole



Supplemental Figure S3

Progressive sealing of the efferent arteriole correlates with decreasing effective filtration flux. A-B. The glomerular endothelium shows a specific sequence of morphological changes towards the efferent arteriole (first described in [3]): 1. Open fenestrae (A', dotted line), 2. transition to diaphragmed fenestrae within the same endothelial cell (A', continuous line, B, arrows). 3. Finally, endothelial cells become continuous and filtration is prevented (arrowheads in B). **B,** Schematic of endothelial changes (as described in [3]). Note that changes in endothelial morphology are independent of podocyte foot process formation (A', white arrowheads). **C. Schematic of the pressure profiles** correlate with endothelial morphology along a filtering fenestrated capillary (not to scale). Towards the distal end, effective filtration flux is reduced by the increasing oncotic pressure of the retained plasma proteins (termed *filtration pressure reduction* or *equilibrium*).

Mathematical Model for VEGF backfiltration

Following the electrokinetic model [4], we assume that VEGF backfiltration is governed by the transport mechanisms of diffusion, convection, and electromigration (also known as electrophoretic effects). The total flux (J) of VEGF is thus given by the sum of a diffusive (J_D), a convective (J_C), and an additional electrophoretic (J_E) flux, i.e.,

(1) $J(x) = J_D(x) + J_C(x) + J_E(x)$,

where x in (0,L) denotes the spatial position within the filter and L is the thickness of the filter. Since VEGF does not accumulate within the renal filter, mass conservation yields that

(2) $J(x) = \text{const.}$, or equivalently $d/dx\, J(x) = 0$.

Denoting the concentration of VEGF in the plasma across the filter by C(x), the diffusive flux reads

(3) $J_D(x) = - D_0 H_D\, d/dx\, C(x)$,

where D_0 is the diffusion coefficient determined by the Stokes-Einstein equation assuming a spherical particle diffusing through a liquid medium at a low Reynolds number,

(4) $D_0 = K_B T /(6 \pi \eta r_{VEGF})$.

Here, K_B is Boltzmann's constant, T is the temperature (37°C = 310.15K), η is the viscosity of the solvent, and r is the Stokes radius of the molecule.

The Stokes-Einstein relation does not account for the pore-structure of the membrane with pores that are from its size similar to the VEGF molecule. As such, the standard diffusion coefficient generally overpredicts mass transport. An analytical model for pore-diffusion (see Equ. (16) in [5]) is used to estimate hindrance factors H_D that account for the diffusive transport in small pores, with

$$H_D = 1 + 9/8\ \lambda \ln(\lambda) - 1.56034\ \lambda + 0.528155\ \lambda^2 + 1.91521*\lambda^3 - 2.81903*\lambda^4 + 0.270788*\lambda^5 + 1.10115*\lambda^6 - 0.435933*\lambda^7,$$

where λ is the particle-to-pore ratio ($\lambda = r_{VEGF} / r_{pore}$).

The convective flux is directly proportional to the velocity and given by

(5) $$J_C(x) = H_C\ v_{H2O}\ C(x),$$

where H_C is the hindrance factor for convection that accounts again for the pore structure of the membrane (taken from [5] Equ. (18)), given by

$$H_C = (1-\lambda)^2\ (1 + 3.867\ \lambda - 1.907\ \lambda^2 - 0.834\ \lambda^3) / (1 + 1.867\ \lambda - 0.741*\lambda^2).$$

The flow velocity of the solvent across the filter v_{H2O} is proportional to the pressure difference.

Finally, the electrophoretic flux is given by

(6) $$J_E\ (x) = H_E\ \mu_E\ E\ C(x),$$

where $E = \Delta\Phi/L$ is the electric field, with $\Delta\Phi$ denoting the electric potential difference, $\mu_E = q / (6\ \pi\ \eta\ r_{VEGF})$ is the electrophoretic mobility, and $q = z_{VEGF}\ e$ is the charge of the molecules with valence z and elementary charge e. An analytic approximation for the hindrance factor of the electrophoretic flux is not given in literature[5]. In agreement with previous works, we assume that the hindrance factor for the electrophoretic flux is equivalent to the one for convection ($H_E=H_C$). A detailed discussion on this assumption is given in[4, 6].

For the case that the electric field results from the streaming potential, the strength of the electric field E depends on the velocity v_{H2O}. The streaming potential in a capillary of radius r_{pore} in a dielectric material can be estimated on an analytical basis (*see Electrochemical Systems, Third Edition, by John Newman and Karen E. Thomas-Alyea, Wiley Interscience*) and is given by

(7) $E_{str} = -8 v_{H2O}\, \zeta \varepsilon / (r_{VEGF}^2 \kappa_{eff})\, I_2(r_{pore})/I_1(r_{pore})$,

where ζ is the streaming potential, ε is the dielectric constant of the liquid, κ_{eff} the effective electric conductivity of the electrolyte, and $I_2(r_{pore})/I_1(r_{pore})$ denote the modified Bessel function of the first kind of order one and two in dependence of the pore-radius r_{pore}. As most of the coefficients in this equation are unknown, we only make use of the general linear relationship between the streaming potential and the velocity v_{H2O}. From eq. (7), the known potential difference of -0.2 mV at a glomerular filtration rate of GFR = 180 liters/day, and the relation v_{H2O} = GFR / A_{Filter} with A_{Filter} being the filter surface area we approximate the electric field with

(8) $E_{str} = c_1\, v_{H2O}$,

where the constant c_1 yields $-3.2*10^8$ with unit Vs/m².

A first order differential equation for the unknown concentration, C(x), is obtained if the different flux contributions (eqn. 3,5, and 6) are used in equation (2), which yields

(9) $(H_C + H_E\, \mu_E\, c_1)\, v_{H2O}\, d/dx\, C(x) = d/dx(D_0\, H_D\, d/dx\, C(x))$

This is the classical diffusion-advection equation that can be rewritten in dimensionless form as

(10) $Pe_{C+E}\ d/dx\, C(x) = d/dx(d/dx\, C(x))$

With the Peclet number relates all fluxes proportional to the velocity (convection and electromigration) to the diffusive fluxes

(11) $Pe_{C+E} = (H_C + H_E\, \mu_E\, c_1)\, v_{H2O} / D_0\, H_D$

The boundary conditions behind the filter is

(12) $C(L) = C_{Pod}$

Here, C_{Pod} denotes the concentration of VEGF at the level of the podocytes. Note that the concentration C_{Pod} can take any value because the solution is linear dependent on

this boundary condition. The sieving coefficient is independent from the choice of $C_{Pod.}$

The second boundary condition is connected to the VEGF concentration in the capillaries which rises from C_{inlet} to $C_{outlet.}$ At any position y in the capillary, the concentration is given by $C_{capillary}(y)$ such that the boundary condition in front of the filter yields

(13) $C(0,y) = C_{capillary}(y)$

The differential equation describing the increase in VEGF in the capillary along the y-coordinate is given later. The boundary conditions (12) and (13) allow to define a dimensionless concentration $\Phi=[C(\Xi)-C(0)]/[C(L)-C(0)]$, where Ξ is the dimensionless coordinate $\Xi = x/L$. The differential equation has the solution

(14) $\Phi = [\exp(Pe_{C+E}\ \Xi) - 1]/\exp(Pe_{C+E} - 1)$.

With the given concentration, the total flux of VEGF in front of the filter (x=0) is

(15) $J(x=0,y) = (H_C + H_E\ \mu_E\ c_1)\ v_{H2O}\ C(0,y) - D_0\ H_D\ Pe_{C+E}\ /L/(\exp(Pe_{C+E})-1)\ (C_{pod}-C(0,y))$

In the two-dimensional model, the total flux of VEGF contributes to an increase in VEGF concentration in the capillary. The following differential equation describes the process:

$dC/dy\ v_{capillary}\ \pi\ r^2_{capillary} = -J(x=0,y)\ 2\pi\ r_{capillary}$.

The negative sign states that the flux J(0) leaves the control volume of the capillaries and results from the definition of x. The VEGF concentration in the systemic circulation is negligible [1]) such that the boundary condition at the inlet of the capillary reads $C(y=0) \approx 0$.

From the definition of the individual fluxes $J_i(x)$ (i = D, C, E) and the solution (14) we can compute the averaged fluxes in front of the filter defined by

(15) $J_{av} = 1/H \int_0^{Lcapillary} J(x=0,y)\ dy$.

In a similar way, the different flux contributions $J_{D,av}$, $J_{C,av}$, and $J_{E,av}$ can be calculated in front and also behind ($x=L_{filter}$) the filter.

Glossary

A_{filter} filter surface [m^2]

C1 constant decoupling the electric field E from the velocity v_{H2O} [Vs m^{-1}]

C_{pod} concentration of VEGF at the podocyte

$C_i(x)$ concentration of VEGF [mol m^{-3}] across the length x of the filter

D_0 diffusion coefficient [m^2 s^{-1}]

$\Delta\Phi$ potential difference [V]

e elementary charge 1.6eE-19 [C]

ε dielectric constant of the liquid [F m^{-1}]

E electric field strength [V/m^{-1}]

η eta, viscosity [Pa s]

Φ dimensionless concentration [-]

GFR glomerular filtration rate [if not denoted otherwise: liters/day]

H_C hindrance factor for convection [-]

H_D hindrance factor for diffusion [-]

J_i flux i of VEGF across the filtration barrier [mol s^{-1} m^{-2}]

K_B Boltzmann's constant [J/K]

L length (thickness) of glomerular filter [m]

Pe_{C+E} Peclet number including convection and electrophoretic mobility

q charge of a molecule, q = z e

r_{pore} radius of pore [m]

r_{VEGF} effective Stokes-Einstein radius of solute [m]

θ_{alb} sieving coefficient ($urine_{albumin}$ * $perfusate_{inulin}$) / ($serum_{albumin}$ * $urine_{inulin}$) [dimensionless]

T temperature [K]

μ_E electrodynamic mobility [m^2/s]

v_{H20} velocity of solute [m/s]

$v_{capillary}$ plasma velocity in the capillary [$m\ s^{-1}$]

x coordinate in the direction of the filter [m]

y coordinate in the direction of the capillary [m]

z_{VEGF} valence of a molecule indicating its charge [dimensionless number]

ζ zeta potential [V]

Ξ dimensionless coordinate [-]

Numerical Parameter Values

A_{filter}	1 m^2 (estimated from [7])
c_1	$-1/9*10^{-5}$ V*d/l
e	$1.602*10^{-19}$ C
η	10^{-3} Pa*s
K_B	$1.3806504*10^{-23}$ J K^{-1}
L	$0.3*10^{-6}$ m
$L_{capillary}$	$26.3*10^{-6}$ m (estimated from [7])
r_{pore}	$4.18*10^{-9}$ m
r_{VEGF}	$2.7*10^{-9}$ m
T	310.15 K

$v_{capillary}$	$104*10^{-6}$ m s^{-1}
z	10

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