Glomerular Capillary Repair in Thy1.1 Nephritis: Identification of Underlying Mechanisms and Molecular Players



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Structure and Formation of Blood Vessels



Hendrix MJC et al. Nature Reviews Cancer 2003, Lafleur MA et al., Expert Reviews in Molecular Medicine 2003

Sprouting: Classical Angiogenesis



Sprouting:

- initiation with proteolytic degradation of the basement membrane
- increased vascular permeability, endothelial cell proliferation and migration
- * maturation with pericyte recruitment

Intussusception: Alternative Mode to Sprouting Angiogenesis





Visualization of intussuception: three-dimensional reconstruction of vasculature with adequate resolution



vascular casting with scanning electron microscopy

tiny holes = pillar formation = intussusception

Burri et al., Dev Dynamics 2004

Sprouting versus Intussusceptive Angiogenesis

	sprouting	intussusception
Endothelial cell proliferation	n high	low
Permeability	high	low
Duration	days	hours
Level of invassiveness	high, sprouts can invade avascular regions and bridge vascular gaps	low, but can expand existing capillary networks

Intusussceptive angiogenesis has rather modest requirements and thus is particularly suitable as angioadaptive and healing mechanism

The Role of Intussusceptive Angiogenesis

Intussusception was described in capillaries, but also in small veins and arteries



Physiology: embryonic avian kidney and lung, development of chick CAM; in mammary gland (Makanya A 2005 and 2007, Schlatter P 1997, Djonov V 2001).

Pathology: the switch from sprouting to intususceptive angiogenesis occurs during tumor recovery from anti-angiogenic treatment or radiotherapy (Hlushchuk R 2008, Semela D 2007)

Molecular Factors Regulating Angiogenesis



Proliferation

sprouting



VEGF receptor / VEGF FGF receptor / FGF

Grandient in growth factor distribution?



PDGF receptor / PDGF Tie-2 receptor / angiopoietins Eph receptor / ephrins

Targeting/Maturation

intussusception



Hemodynamics -shear stress

The Glomerulus: the Capillary Tuft with Unique Morphology and Functions



glomerular filtration barrier

glomerulus

Angiogenesis in Kidney Diseases



 \checkmark Pro- and anti- angiogenic factors are widely expressed in healthy kidney.

 \checkmark Several acute and chronic renal diseases were found to be associated with the disturbed expression of angiogenic factors.

 \checkmark There is an emerging concept that manipulation of angiogenic response can attenuate the disease process.

However, little is known about the mechanisms by which glomeruli spontaneously recover from injury.

Aim of the Study

>Which angiogenic mode is responsible for capillary recovery from acute mesangioproliferative glomerulonephritis (GN)?

sprouting and/or intusussceptive angiogenesis?

Methods

Electron microscopy:

-scanning electron microscopy (SEM) on vascular casts -SEM on kidney dried at critical point -transmission electron microscopy (TEM)

Histology: -toluidin blue staining on semithin sections

Immunostaining and Western blot methods

Stereology:

-quantification of glomeruli number in the kidney -quantification of podocyte number per glomerulus



Thy1.1 nephritis:

Primary injury to mesangial cells, secondary to endothelium.

*Research has focused on endothelial and mesangial regeneration upon nephritis induction.

*The data concerning the podocyte reaction to the capillary collapse are scarce.

Restoration of Glomerular Capillaries During Thy1.1 Nephritis



10µm

50µm

Arrowhead: tiny hole Arrow: small capillary loop

sprouts were not observed

Restoration of the Glomerular Structure During Thy1.1 Nephritis



Aim of the Study

>Which angiogenic mode is responsible for capillary recovery from acute mesangioproliferative glomerulonephritis (GN)?

 \rightarrow Y mostly intusussceptive angiogenesis

>What is the role of VEGF in intussusceptive angiogenesis and restoration of the glomerular structure during Thy1.1 nephritis?

VEGF Receptor / VEGF Ligand



VEGFR/VEGF system is the most studied pro-angiogenic pair of receptor tyrosine kinase and ligand. VEGFR2 expressed by ECs exerts potent proliferative and prosurvival properties.

Blocking strategies of VEGFR/VEGF are used in anti-cancer therapy.

VEGFR/VEGF in Kidney Pathophysiology



VEGFR2/VEGF are expressed by the tubular epithelium and in the glomeruli

Cohen EP 2010

VEGFR/VEGF signalling leads to worsening of disease in diabetic nephropathy

VEGFR/VEGF signalling leads to disease attenuation in mesangioproliferative glomerulonephritis or thrombotic microangipathy

VEGFR/VEGF has no effects on kidney function in minimal change nephropathy

→ different ways to manipulate VEGFR/VEGF axis, different animal models used, different end points

Upregulation of glomerular VEGFR2/VEGF during the Course of Thy1.1 Nephritis



PTK787/ZK 222584, an inhibitor of VEGFR/PDGFrs (Wood J. et al., Cancer Res 2000)



The Dynamics of Intussusceptive Angiogenesis after PTK/ZK/vehicle Administration



The Dynamics of Intussusceptive Angiogenesis after PTK/ZK/vehicle Administration

PTK/ZK-treated nephritic rats



Impact of PTK/ZK on Glomerular Recovery from Thy1.1 Nephritis

Bc

Bc



Summary and Outlook

Intussusception, not sprouting, is the angiogenic mode responsible for the capillary recovery during Thy1.1 nephritis.

*Loss of nephrons during Thy1.1 nephritis is compensated by the increase of glomerular volume.

Thy1.1 nephritis - associated intussusceptive angiogenesis is largely VEGF - independent.

*VEGF inhibition during Thy1.1 nephritis leads to the downregulation of mesangial α SMA expression, rupture of microaneurysms and presence of microbleedings.

<u>Clinical relevance of the findings</u>: Cave VEGF inhibition in patients with current of a history of glomerulonephritis!

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