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

Uyen Huynh Do, MD, MME
Department of Nephrology and Hypertension
University Hospital Berne
Switzerland

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1. Introduction
   - Formation of new vessels and its regulation
   - Morphology and functions of the glomerulus

2. Aim of the study

3. Results
   - Glomerular repair by intussusceptive angiogenesis in Thy1.1 nephritis - effects of VEGF/PDGF r inhibition

4. Conclusions

5. Acknowledgements
Structure and Formation of Blood Vessels

artery

endothelial cell

basement membrane

adventitia

media

capillary

lumen

endothelial cell

pericyte

basement membrane

vasculogenesis

angioblasts

angiogenesis

sprouting

intussusception

Sprouting: Classical Angiogenesis

- initiation with proteolytic degradation of the basement membrane
- increased vascular permeability, endothelial cell proliferation and migration
- maturation with pericyte recruitment
Visualization of intussuception: three-dimensional reconstruction of vasculature with adequate resolution

血管铸造与扫描电子显微镜

tiny holes = pillar formation = intussusception

Burri et al., Dev Dynamics 2004
# Sprouting versus Intussusceptive Angiogenesis

<table>
<thead>
<tr>
<th></th>
<th>Sprouting</th>
<th>Intussusception</th>
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</thead>
<tbody>
<tr>
<td><strong>Endothelial cell proliferation</strong></td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td><strong>Permeability</strong></td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>days</td>
<td>hours</td>
</tr>
<tr>
<td><strong>Level of invassiveness</strong></td>
<td>high, sprouts can invade avascular regions and bridge vascular gaps</td>
<td>low, but can expand existing capillary networks</td>
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Intussusceptive 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


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

**Proliferation**

- VEGF receptor / VEGF
- FGF receptor / FGF

**Targeting/Maturation**

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

**Hemodynamics**

- Shear stress

Burri et al., Dev Dynamics 2004
The Glomerulus: the Capillary Tuft with Unique Morphology and Functions

- **podocyte**
- **glomerulus**
- **glomerular filtration barrier**
- **capillary lumen**
- **urinary space**
- **endothelial cells**
- **mesangium**
- **GBM**

**Diagram:**
- Afferent and Efferent arterioles
- Podocyte
- Mesangium
- Urinary space
- Capillary lumen lined with endothelial cells
- Glomerulus
- Glomerular filtration barrier
Angiogenesis in Kidney Diseases

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

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

- 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 intussusceptive 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
Experimental Model: Rat Thy1.1 Nephritis

**Time course**

- **D0**: Tail vein injection of 1 mg/kg b.w. of anti-Thy 1.1 ab
- **D3 – D12**: Thy 1.1 Nephritis
  - Capillary destruction
  - Mesangial expansion
- **D14 – D21**: Spontaneous recovery

**Histology**

- **ctrl**
- **D5**
- **D9**
- **D14**

**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

Star: microaneurysm
Arrowhead: tiny hole
Arrow: small capillary loop

sprouts were not observed
Restoration of the Glomerular Structure During Thy1.1 Nephritis

**Intussusception**

- Numerical density per vessel area
- Tiny holes (<2μm)
- Small capillary loops (2-10μm)

**Day of nephritis**
- ctrl
- D5
- D9
- D14
- D21

**Glomerular Volume**

- Glomerular volume x 1000μm³
- ctrl
- D5
- D9
- D14
- D21

**Number of glomeruli**

- Glomeruli number x 10³
- Ctrl
- D21
Aim of the Study

- Which angiogenic mode is responsible for capillary recovery from acute mesangiproliferative glomerulonephritis (GN)?

  ⇒ Y mostly intussusceptive angiogenesis

- What is the role of VEGF in intussusceptive angiogenesis and restoration of the glomerular structure during Thy1.1 nephritis?
VEGFR/VEGF system is the most studied pro-angiogenic pair of receptor tyrosine kinase and ligand. VEGFR2 expressed by ECs exerts potent proliferative and pro-survival properties.

Blocking strategies of VEGFR/VEGF are used in anti-cancer therapy.
VEGFR/VEGF signalling leads to worsening of disease in diabetic nephropathy

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

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

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

PTK/ZK-treated nephritic rats

Day 5

Day 9

Day 14

vehicle-treated nephritic rats
The Dynamics of Intussusceptive Angiogenesis after PTK/ZK/vehicle Administration

PTK/ZK-treated nephritic rats

Day 5

Day 9

Day 14

density of tiny holes

**

Day of nephritis

ctrl D5 D9 D14

Day of nephritis

density of small capillary loops

*

Day of nephritis

ctrl D5 D9 D14
Impact of PTK/ZK on Glomerular Recovery from Thy1.1 Nephritis

(a) Bar graph showing the impact of PTK/ZK on glomerular recovery from Thy1.1 nephritis. The graph compares the vehicle and PTK/ZK groups at different time points (ctrl, D5, D9, D14, D21).

(b) Bar graph showing the percentage of microaneurysms. The graph compares the vehicle and PTK/ZK groups at Day 5.

(c) Western blot analysis of αSMA, β-actin, Thy1.1, and β-actin in the control, vehicle, and PTK/ZK groups.

(d) and (e) Micrographs showing glomerular structure in the control group (d) and the vehicle group (e) at Day 5.

(f) and (g) Micrographs showing glomerular structure in the PTK/ZK group (f) and a larger view at Day 5 (g).

(h) and (i) Micrographs showing glomerular structure in the PTK/ZK group (h) and a larger view at Day 5 (i).
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 αSMA expression, rupture of microaneurysms and presence of microbleedings.

**Clinical relevance of the findings:** Cave VEGF inhibition in patients with current of a history of glomerulonephritis!
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