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Eph receptors and the kidneys: New kids in the block ?



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I – The case



M. B., 25 year old male patient





Urine sediment



✓ EC-uria✓ "Glomerular Ec"

✓ RBC casts



Serum creatinine: 250 μmol/l

Mesangio-proliferative Glomerulonephritis



... with IgA depots



IgA nephropathy worldwide the most common glomerulopathy



adapted from Feehally, 2008

IgA nephropathy: A wide variation in the natural history



What makes the difference between resolution and scarring ?





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II – The model





Rat Thy1.1 Nephritis An acute mesangioproliferative GN with spontaneous recovery





Rat Thy1.1 Nephritis



Question 1:

What characterizes early angiogenic repair in Thy1.1 nephritis ?

Which are the main angiogenic factors involved?



Pathways to recovery and loss of nephrons in anti Thy1 GN

Kriz et al, JASN 2003; 14; 1904-26





Day 2: Capillary expansion (yellow), mesangiolysis (blue), but folding of GBM preserved thanks to podocytes. Day 7-9: glomerular lobule, where the folding pattern of GBM is maintained in central portion, niches at turning points of GBM are filled with dark processes of podocytes .



"...Podocytes could prevent immediate expansion, providing time for the repair process to start from a state of moderate damage"



Question 2:

Which factors contribute to podocyte survival in response to mesangial collapse ?

Two types of angiogenesissproutingintussusception







vascular cast and SEM

Endothelial cell proliferation	n high	low
Permeability	high	low
Duration	days	hours
Invasiveness	high, sprouts can invade avascular regions and bridge	low, but can expand existing capillary networks
	vascular gaps	Burri et al. Dev Dynamics 2004

Molecular Factors Regulating Angiogenesis



sprouting



Proliferation

VEGF receptor / VEGF FGF receptor / FGF

Granient in growth factor distribution?



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

intussusception



Flow alternations -shear stress (endothelium)

Targeting/ Maturation

Burri et al. Dev Dynamics 2004

Restoration of Glomerular Capillaries: vascular casts







Glomerular Capillaries repair mainly through Intussception

Q



















Wnuk et al., A J Pathol 2011

VEGF-R2 is strongly upregulated and phosphorylated during Thy1.1 nephritis





The VEGF inhibitor PTK787/ZK influenced intussusceptive angiogenesis only after long-term application



Wnuk et al., Am J Pathol 2011



day of nephritis

In Thy1.1 nephritis, ephrinB2 is upregulated @ days 5-14, while EphB4 is strongly phosphorylated at days 7-9





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III – The "kids"



Erythropoeitin Producing hepatocellular Carcinoma



Erythropoeitin Producing hepatocellular Carcinoma



Eph receptors and ephrins The early years: 1987 - 1999 Pivotal role in developmental processes

- Axonal guidance
- Somite formation
- Embryonic vessel formation

EphB4 / ephrinB2 interaction at boundary regions determine arteriovenous anastomosis



Wang, Cell 1998

Eph receptors and ephrins The later years: 2000 – 2010 Important players in the adult organism

- Pancreas: Insulin signaling
- "Bone homeostasis"
- Intestine: Cell migration along crypt axis

β cells: EphA5 / ephrinA1 regulate insulin secretion in response to glucose



Konstantinova, Cell 2007

β cells: EphA5 / ephrinA1 regulate insulin secretion in response to glucose



Konstantinova, Cell 2007

EphB4/ephrinB2 regulate the balance between bone resorption & formation



Zhao, Cell Metabol 2006

Temporally Compartmentalized Expression of Ephrin-B2 during Renal Glomerular Development JASN 2001; 12: 2673-82

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Eph receptors in the adult kidney EphA2 / EphB2 and "stress response" in renal tubuli

EphA2 is upregulated in tubular epithelium following water deprivation or diet supplementation with urea (Xu, *AJ Physiol* 2005)

EphA2 upregulated in tubular epithelium in ischemiareperfusion injury (Baldwin, *AJ Physiol* 2006)

EphB2 regulates the cytoarchitecture of the medullary tubular epithelial cells (Ogawa, JCS 2006)

In Thy1.1 nephritis, ephrinB2 is upregulated @ days 5-14, while EphB4 is strongly phosphorylated at days 7-9



BHG712 - a potent small molecular weight inhibitor of EphB4 forward signaling *in vivo*

			in vitro kinase assay		
NVP-BHG712			kinase	IC50 (nM)	
			c-raf	395	
			C-SIC	1266	
			c-Abl	1667	
			Tie2	>10 000	
			Axl	>10 000	
			Her1	>10 000	
cell based assays			PKA	>10 000	
RTK	ED50 [nM]		CDK1	>10 000	
EphB4	25 (n=8)		PKB	>10 000	
VEGFR2	4200 (n=3)		PDK1	>10 000	
IGF1R	>10.000 (n=3)		FGFR3	>10 000	
InsR	> 10.000 (n=3)		c-Met	>10 000	

Martiny-Baron G. et al., Angiogenesis 2010

BHG 712 inhibits EphB4 forward signaling in podocytes during Thy 1.1. nephritis



IP: EphB4 veh 3h 6h 24h WB: PTyr WB: EphB4

BHG712 50 mg kg b.w.



No effects of BHG712 on non-nephritic rats



BHG 712 administration to nephritic rats impairs the progress of Intussusceptive Angiogenesis



After BHG712 podocytes show signs of maladaptation

D9/BHG712

Absorption droplets



Pseudocysts

D9/vehicle

Apical openings

After BHG712 podocytes show signs of maladaptation

D9/vehicle

D9/BHG712



Stretching (i), shedding into Bowman space (k) —> Foot process fusion (k)
Endothelium: loss of fenestration (h,k)
Widening of subendothelial space

BHG 712 leads to podocyte loss due to apoptosis



TUNEL eosin







1

EphB4 signaling is activated in response to direct podocyte injury *in vitro* or ephrinB2/Fc

Immortalized podocyte cell line



PAN = puromycin aminonucleoside



Supernatant of stimulated podocytes



WB: VEGF





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IV – Summary





Glomerular repair in Thy 1.1 nephritis

Question 1:

What characterizes early angiogenic repair in Thy1.1 nephritis ?

Early angiogenic repair in Thy1.1 nephritis happens through intussusceptive angiogenesis

VEGF inhibition in Thy1.1 nephritis induces capillary abnormalities but impacts on intussusceptive angiogenesis only after long term treatment

Wnuk et al., Am J Pathol 2011

Podocyte EphB4 signaling regulates intussusceptive angiogenesis necessary for capillar regeneration



Glomerular repair in Thy 1.1 nephritis

Question 2:

Which factors contribute to podocyte survival in response to mesangial collapse ?

 ❖ Podocytes respond to a mesangiolysis by activating EphB4 forward signaling
→ "Survival signal" permitting podocytes to take over the role of mesangial cells and prevent total capillary collapse

→ Dual role of EphB4 receptor during glomerular repair in Thy 1.1 nephritis

Wnuk et al., *KI* 2012





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