The EGF Receptor promotes glomerular injury in necrotizing crescentic glomerulonephritis.

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Actualités néphrologiques
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Glomerulonephritis
12% of ESRD

pauci-immunes RPGN
Activation of neutrophils
In response to ANCA

anti-GBM Glomerulonephritis
anti-α3NC1 / α5NC1 antibodies
Autoreactive T Lymphocytes
Epitope spreading

immune complexes RPGN 25%
Auto-antibodies
Complement activation

Common mechanisms?
Glomerular epithelial cells

IgAN
Vasculitis / ANCA
SLE
Goodpasture / anti-GBM
post infectious GN
Cryoglobulinemia...
Podocytes in NCGN

Up to 50% of the crescentic cells
Transformation of their phenotype:
- loss of differentiation markers
- Migration and proliferation

Proliferation of podocytes induced by the deletion of the \textit{vhl} gene is sufficient to develop a NCGN

\textit{Ding, Nat Med, 2006}
Glomerular parietal epithelial cells in NCGN

- involvement of the GPEC in human and murine crescentic glomerulonephritis
- up to 80% of the crescentic cells
- Progenitor cells:
  - CD24 and CD133 expression (human)
  - podocytes regeneration
ANCA vasculitis

anti-GBM antibodies

Immune complexes

Immunosuppressants

Inflammation injury
- rupture of the glomerular filtration barrier
- leakage of plasma proteins

Local factors?

Proliferation and migration of glomerular epithelial cells

HB-EGF

EGFR

Proliferation
Migration
Inflammation?
Heparin-binding EGF-like growth factor (HB-EGF)

- Growth Factor of the EGF family
- Ligand of the ErbB family (EGFR, ErBb4)

Ligand binding
Hetero or homodimer formation
Autophosphorylation
Recruitment of intracellular signaling pathways

EGF
TGFα
Amphireguline
Epigen

HB-EGF
Epireguline
Betacelluline

Neuregulins

EGFR/ErbB1
ErbB2
ErbB3
ErbB4

PI3K
Ras
MAPK
STAT3, STAT5
HB-EGF expression pattern:
- Epithelial cells
- Vascular SMC
- endothelial cells
- Mesangial cells
- T lymphocytes
- Macrophages
- Cancer cells

Juxtacrine and paracrine activity

Involvement in NCGN?

Adapted from Flamant et al, Med Science, 2005
**Physiological function of EGFR signaling pathways:**

- Mg reabsorption (TRMP6)
- Tubular regeneration

**EGFR pathway in renal diseases:**

- Fibrosis
- Polycystic kidney disease

**Glomerular expression of HB-EGF**

- Diabetes
- FSGS (Puromycin model)
- NTS anti GBM (early induction)

Paizis Kidney Int Feng, JCI 2000

**Mesangial expression**

- anti Thy1.1 GN
- human GN (IgAN, MPGN, SLE)

Takemura J Pathol 1999

Groenestege, Terzi, J Clin Invest, 2007
Zhuang, Am J Physiol Renal Physiol, 2004

Terzi, J Clin Invest, 2000
Richards, J Clin Invest, 1998
Anti GBM nephropathy

- Early and transient induction of HB-EGF
- Early HB-EGF dependant hemodynamic modifications
  (↓ SNGFR prevented with an anti-HBEGF antibody)
Nephrotoxic Serum (NTS)

Sheep IgG (preimmunization)

Sheep IgG

IgG from sheep

NTS

sacrifice

J-5

J1, 2, 3

J10

anti-MBG Glomerulonephritis accelerated model
mRNA of HBEGF is induced in the anti-GBM GN

Bollée & Flamant et al. Nat Med 2011
Histological lesions and functional impact of anti-GBM GN is reduced in HB-EGF deficient mice

Bollée & Flamant et al. Nat Med 2011
HB-EGF deficiency prevents structural modifications of podocytes in the anti-GBM nephropathy

Bollée & Flamant et al. Nat Med 2011
HB-EGF deficiency restricted to hematopoietic cells does not protect against lesions of anti-GBM GN

HB-EGF can be produced by hematopoïetic cells:
- macrophages
- lymphocytes T

Blotnick, *Proc Natl Acad Sci USA*, 1994

EGFR phosphorylation in glomerulus during anti-GBM GN in mice

Bollée & Flamant et al. Nat Med 2011
EGFR inhibition (AG1478) decreases the severity of anti-GBM GN

Bollée & Flamant et al. Nat Med 2011
HB-EGF deficiency and AG1478 treatment reduces lymphocyte T infiltration in the kidney

CT vehicle AG1478

CD3+ Area in Renal Cortex

CT vehicle AG1478

Bollée & Flamant et al. Nat Med 2011
Erlotinib infused 4 days after NTS injection prevented anti-GBM GN

Bollée & Flamant et al. Nat Med 2011
Epiregulin (KO) or TGF-alpha (wa1) deficient mice are not protected from glomerular lesions or functional alterations in the antiGBM GN model

Lack of epiregulin or TGF-α induction in NCGN

Bollée & Flamant et al. Nat Med 2011
Inducible deletion of *Egfr* in podocytes

Pod-Tet on-Cre *Egfr* \(^{\text{loxP/loxP}}\)

Podocin-rtTA \(\text{TetO Cre} \)

X

+ Doxycycline

**Pod-Tet on-Cre *Egfr* \(^{\text{wt/wt}}\)**

**Pod-Tet on-Cre *Egfr* \(^{\text{loxP/loxP}}\)**

EGFR

Synaptopodin

Merge

*Bollée & Flamant et al. Nat Med 2011*
Specific deletion of EGFR in podocytes attenuates anti-GBM GN in mice

Bollée & Flamant et al. Nat Med 2011
Role of ErbB4 in the RPGN?

Constitutive invalidation of ErbB4 in podocytes
ErbB4 inhibition in podocytes does not modify renal damage and functional alteration of anti-GBM GN
Mouse → Harvest kidney → Sieving

Sieving:
- Ø 100µm
- Ø 40µm

Results:
- Decapsulated Glomeruli (90% of total)
- Capsulated Glomeruli (10% of total)

7 days

Transfer into plates

Expand podocytes from glomeruli

Proliferation assay
Podocyte outgrowth measurement
Role of HB-EGF in the proliferation and migration of podocytes *in vitro*

*Bollée & Flamant et al. Nat Med 2011*
Induction of HB-EGF expression in human crescentic GN

pauci-immune  endocarditis  pauci-immune

Cr  Po  Cr  Po  Po  Pec

pauci-immune  pauci-immune  pauci-immune
Perspectives

- Goodpasture syndrome
- Lupus
- Pauci-Immune
- Endocarditis
- ...

Flamant M et al., Nephrol Dial Transplant 2012
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EGFR phosphorylation in glomeruli is not induced after LPS infusion