New mechanisms and therapeutic options for renal fibrosis – what have we learnt from our research?

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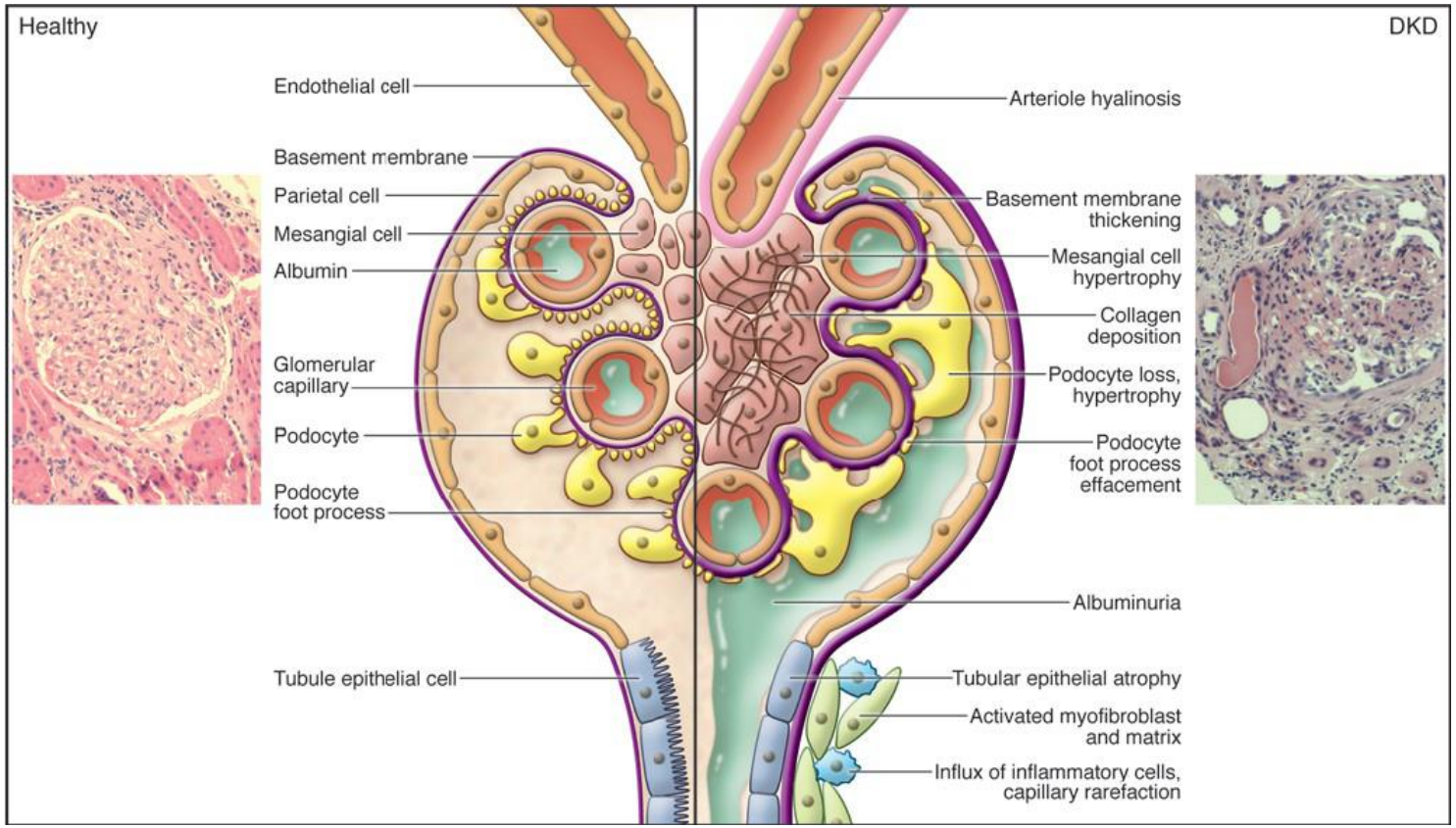
SEMMELWEIS EGYETEM 1769

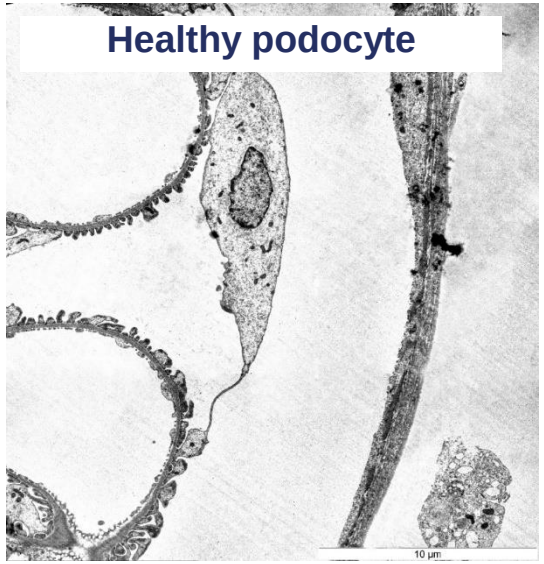
Background - significance

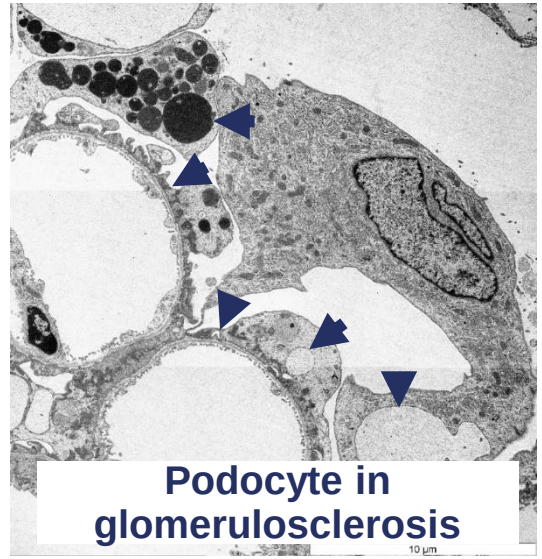
- The number of patients with CKD and renal failure is steadily increasing worldwide (7% ↑ per year), with heterogeneous etiology.
  - Major causes: **diabetes**, hypertension
- During pathogenesis, **renal fibrosis** develops → kidney failure (Th: Tx, dialysis)
- Exact pathomechanism of early fibrotic events remain unclear, this **hampers early detection and effective therapy**
- **No therapeutic breakthrough** since the introduction of RAAS inhibition
- Better understanding of molecular pathomechanisms is needed to develop both more sensitive biomarkers and new targeted therapies.

Main renal lesions in diabetes



Journal of Clinical Investigation, 2014; 124(6), 2333-2340





5000x, TEM (rat kidney) *Kökény G. 2006*

Pathomechanism – what we know I.

- In CKD, **podocytes** are the main victims of glomerular damage
  - Podocyte injury: apoptosis, foot process effacement and detachment from GBM
  - Unable to proliferate under physiological conditions
  - Loss of filtration barrier - proteinuria

- **Oxidative stress,** eg. in DM
  - Reduced eNOS activity
  - Damage of NO signaling

- Increased **RAAS** activity is present
  - (Pro)renin induces TGF-β synthesis
  - AngII via AT1R, aldosteron via MR have pro-fibrotic effects

Pathomechanism – what we know II.

- **TGF-β** is the main player in kidney fibrosis:
  - Activates fibroblasts, increase collagen synthesis and tissue metalloprotease inhibitors (TIMP)
  - TGF-β induced **TIMP-1 overproduction** is characteristic for renal fibrosis,
    - TIMPs inhibit extracellular matrix degradation
  - TGF-β enhances the production and activity of several **transcription factors,**
    - Early growth response-1 (EGR1), JUN/FOS (AP-1 complex), STAT3
  - TGF-β and kidney fibrosis **reduces PPARγ** activity, altering glucose/lipid metabolism of the cells,
  - Induces pro-fibrotic **miRNAs**,
  - Disrupts **autophagy**,
  - Reduces **medullary sodium transport**, dysfunctional osmoregulation.

- Individual progression rates vary in clinical settings despite the same underlying disease, indicating the possible role of **genetic factors**

Podocytes and the NO-cGMP axis

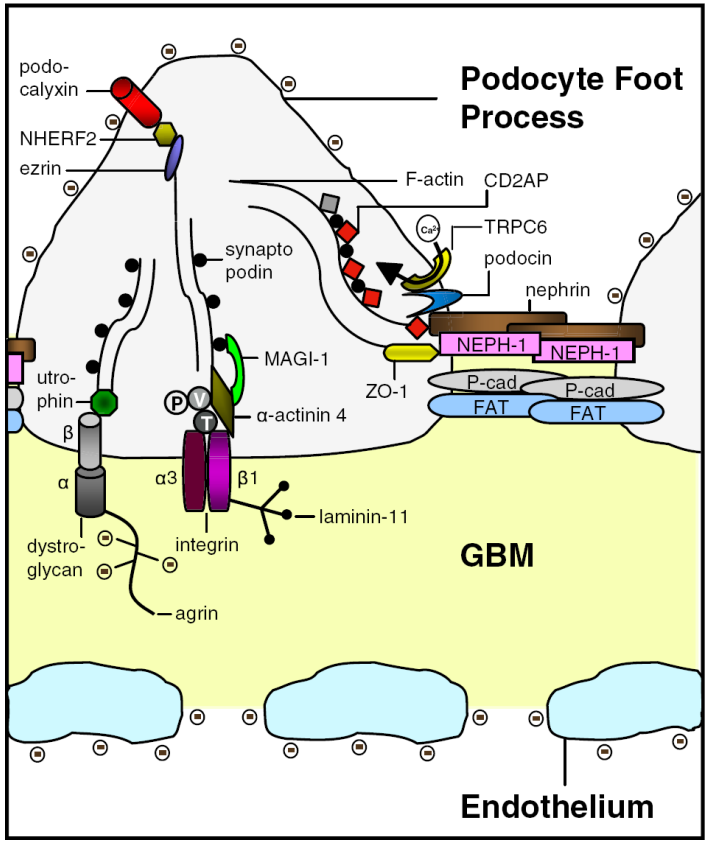
Dr. habil. Gábor Kökény,
associate professor

Podocyte damage

- Increased glomerular pressure, hyperfiltration → shear stress
- Increase in free radicals **(oxidative stress)**
- Reduced **nephrin** and podocin production
- Loss of GBM negative charge
- Changes in GBM structure
- Apoptosis, podocyte loss (GBM denudation)

↓

Proteinuria



Eur J Pediatr (2009) 168:1291–1304

NO-cGMP axis in the kidney

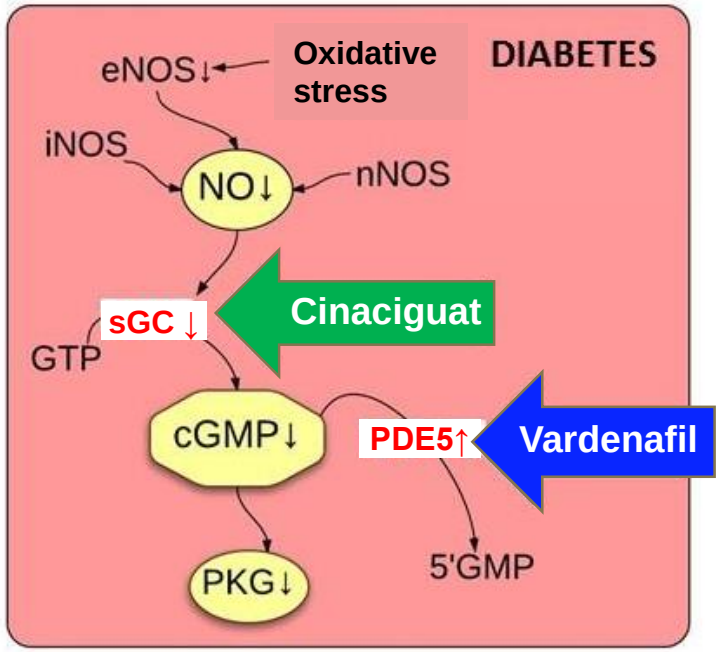
Regulates:

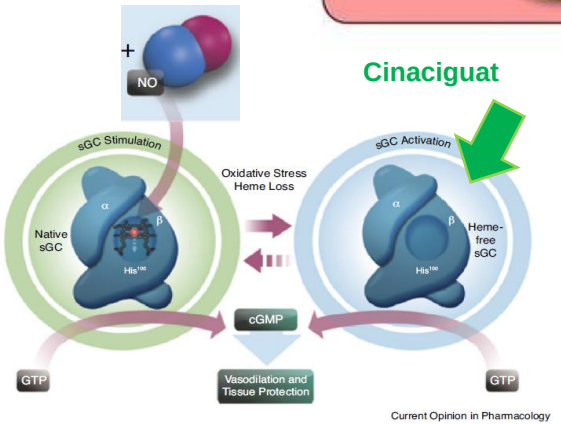
- Glomerular filtration
  (**podocyte** cytoskeleton, slit membrane)
- Glomerular perfusion (afferent arterioles)

In diabetes: oxidative stress → NO-cGMP↓

- eNOS activity ↓
- Function of oxidized sGC↓ – cGMP↓
- Activity of cGMP-degrading PDE5↑

- Intervention: to enhance cGMP signaling





The effect of NO-cGMP on proteinuria

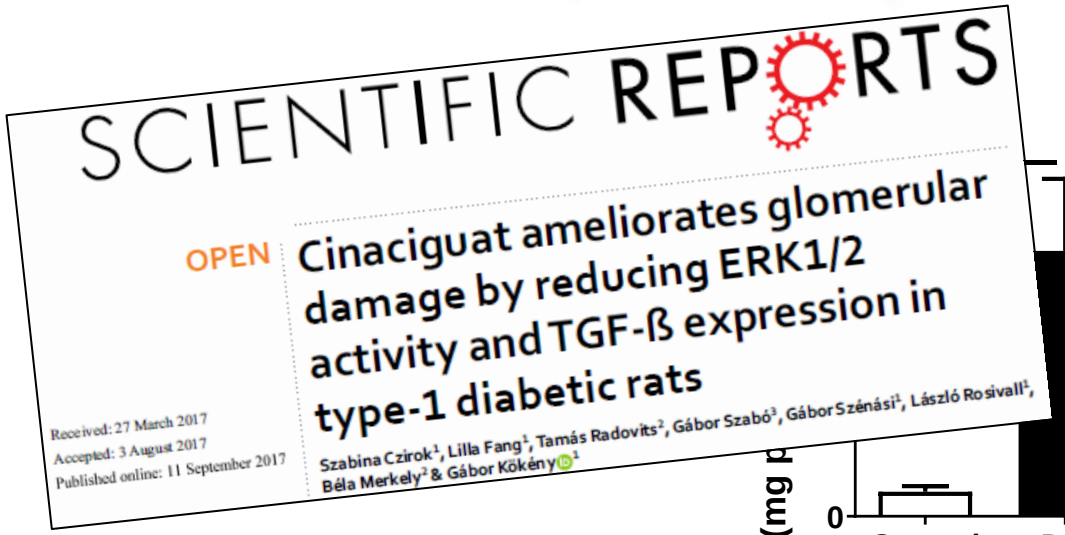
T1DM

\+

sGC activator
Cinaciguat p.o.
(Cin)

or

PDE5 inhibitor
Vardenafil p.o
(Vard)



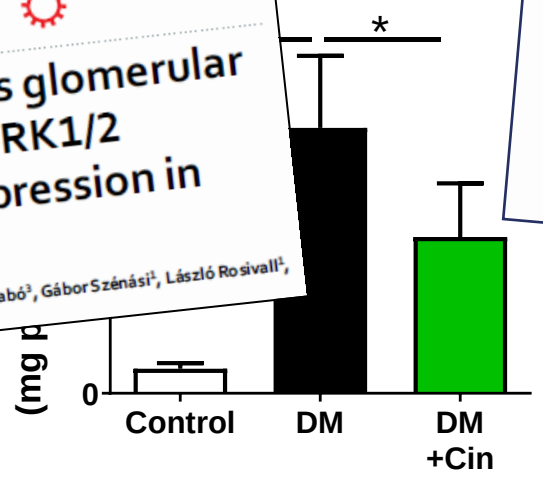
SCIENTIFIC REPORTS

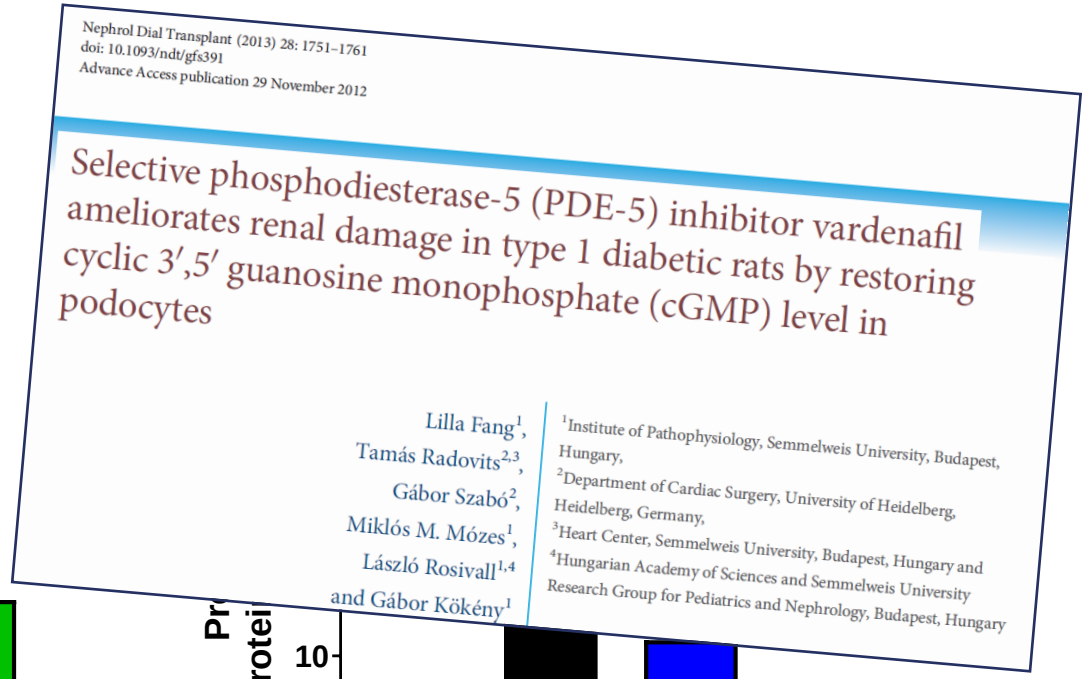
OPEN

Cinaciguat ameliorates glomerular damage by reducing ERK1/2 activity and TGF-ß expression in type-1 diabetic rats

Received: 27 March 2017
Accepted: 3 August 2017
Published online: 11 September 2017

Szabina Czirok[1], Lilla Fang[1], Tamás Radovits[2], Gábor Szabó[3], Gábor Szénási[1], László Rosivall[1], Béla Merkely[2] & Gábor Kökény[1]



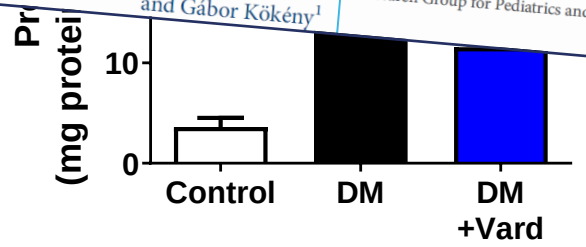


Nephrol Dial Transplant (2013) 28: 1751–1761
doi: 10.1093/ndt/gfs391
Advance Access publication 29 November 2012

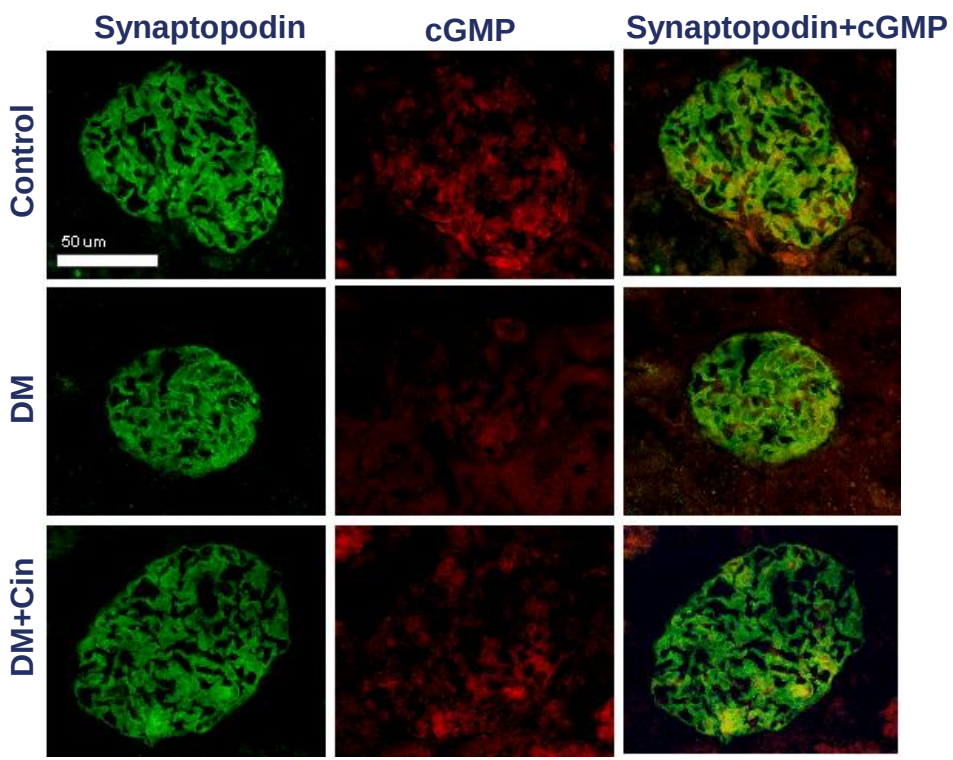
Selective phosphodiesterase-5 (PDE-5) inhibitor vardenafil ameliorates renal damage in type 1 diabetic rats by restoring cyclic 3′,5′ guanosine monophosphate (cGMP) level in podocytes

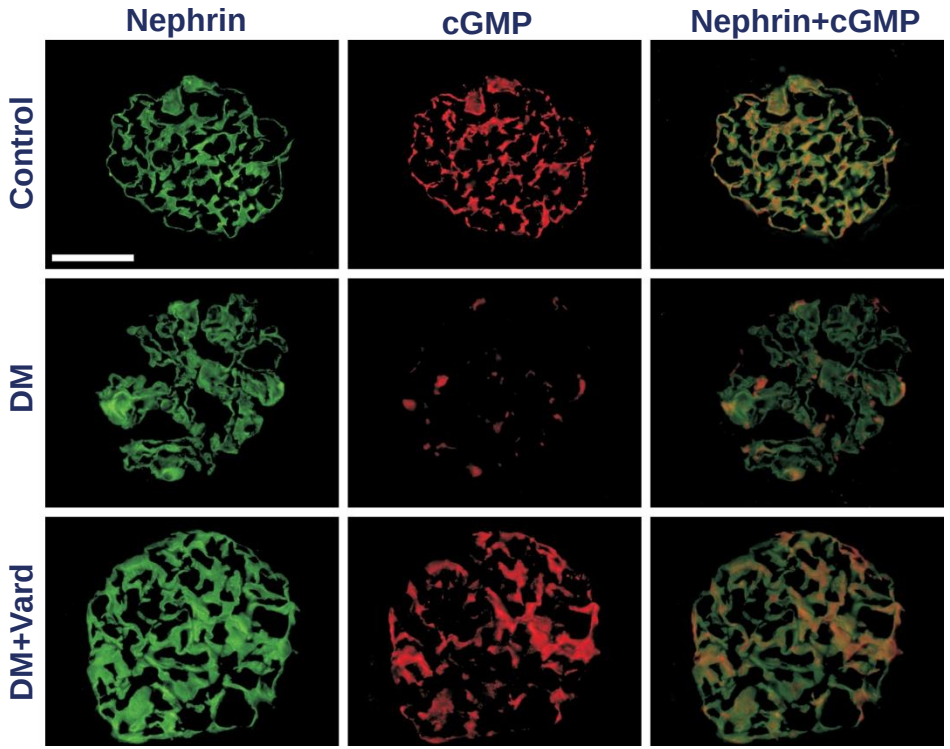
Lilla Fang[1], Tamás Radovits[2,3], Gábor Szabó[2], Miklós M. Mózes[1], László Rosivall[1,4] and Gábor Kökény[1]

[1]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary,
[2]Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany,
[3]Heart Center, Semmelweis University, Budapest, Hungary and
[4]Hungarian Academy of Sciences and Semmelweis University Research Group for Pediatrics and Nephrology, Budapest, Hungary



Cinaciguat and vardenafil increase podocyte cGMP

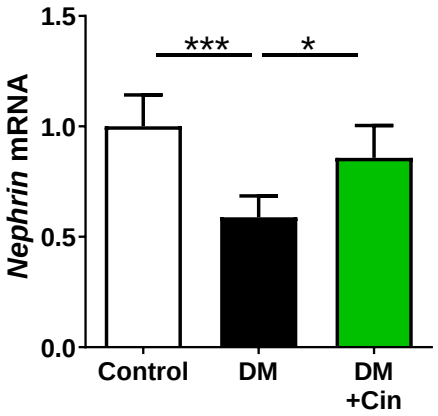


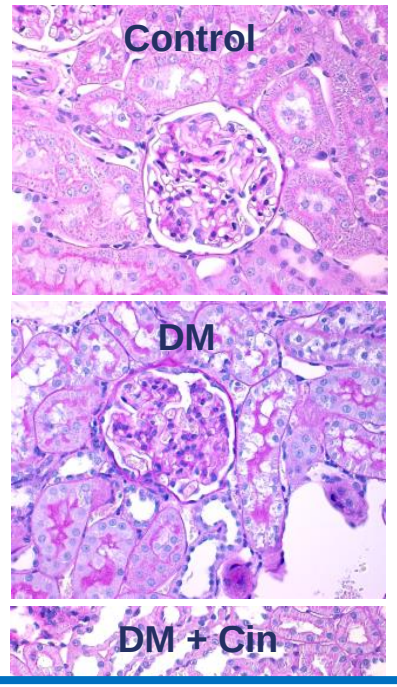


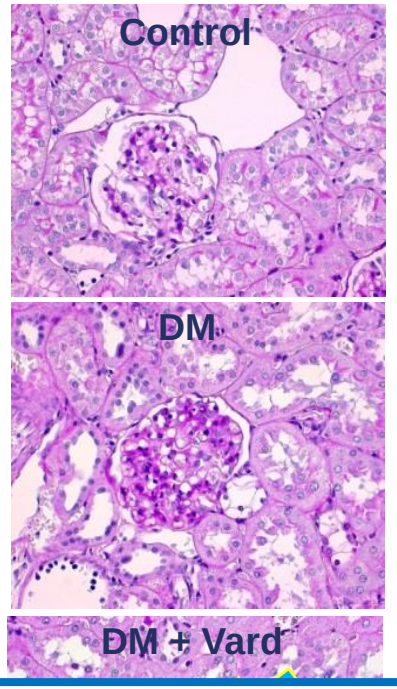
Sci Rep. 2017 Sep 11;7(1):11218.

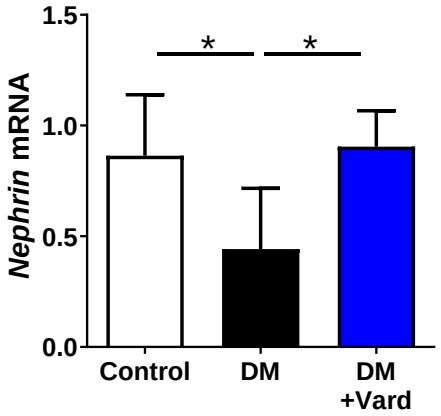
Nephrol Dial Transplant. 2013 Jul;28(7):1751-61.

Enhancing cGMP ameliorated podocyte damage and glomerulosclerosis









In diabetes model both the PDE5A inhibitor and the sGC activator treatment normalized podocyte cGMP levels, reducing fibrosis.

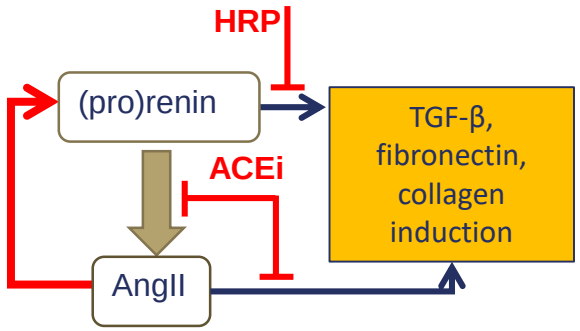
Sci Rep. 2017 Sep 11;7(1):11218.

Nephrol Dial Transplant. 2013 Jul;28(7):1751-61.

Increased RAAS activity: are combination treatments beneficial?

Dr. habil. Gábor Kökény,
associate professor

Effect of (P)RR and ACE inhibition on renin



T1DM

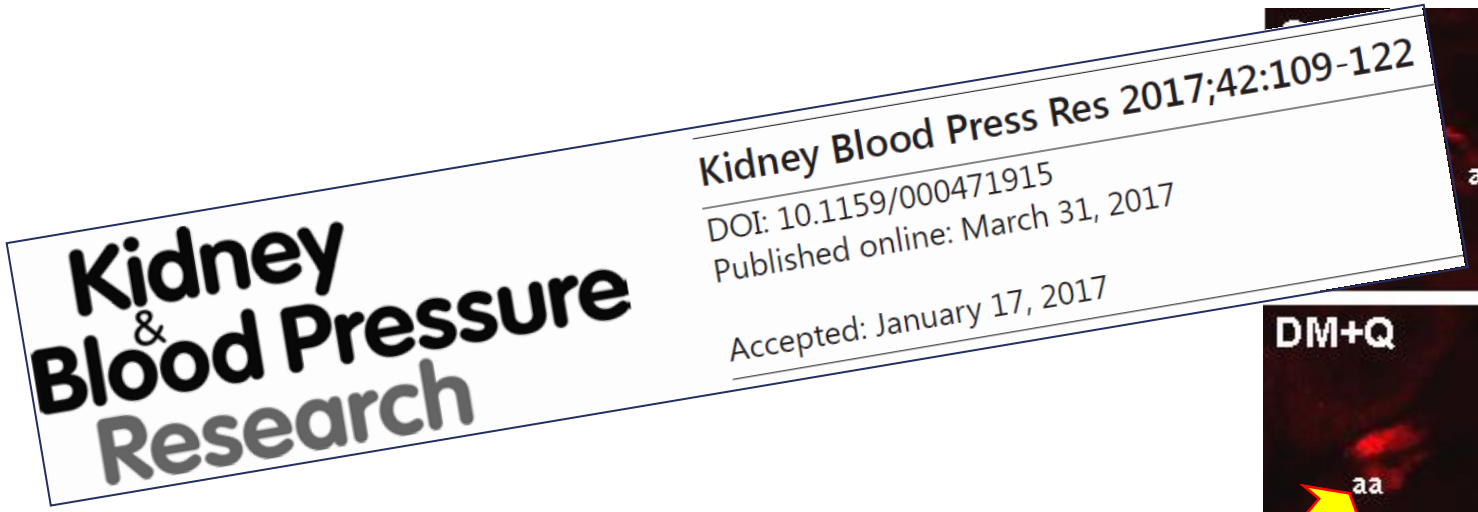
(pro)renin receptor blocking peptide (HRP)

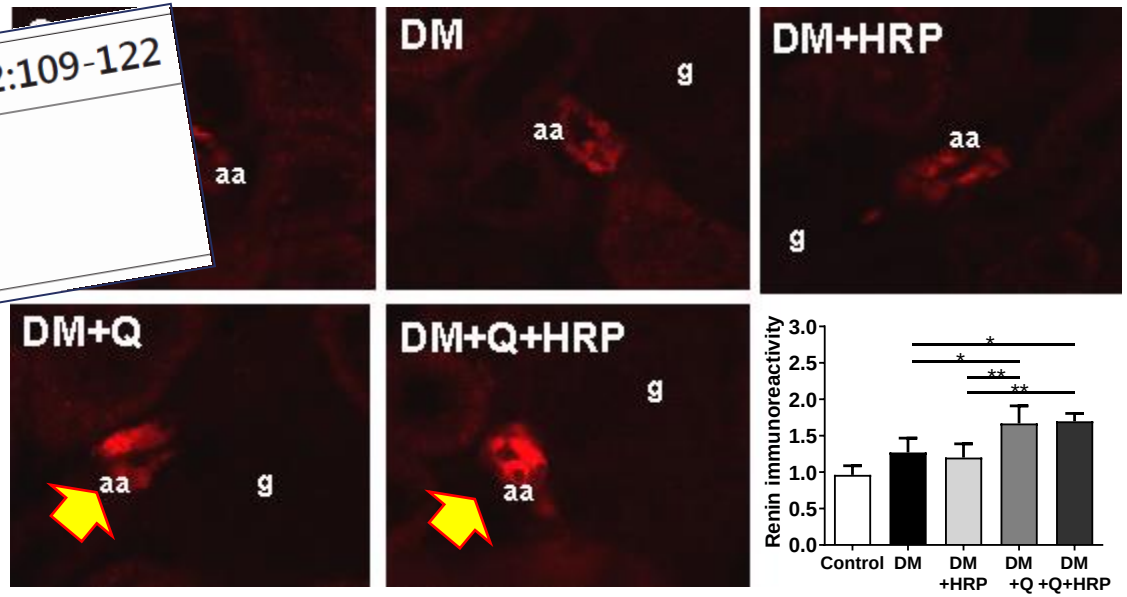
or

ACEi (Quinapril)

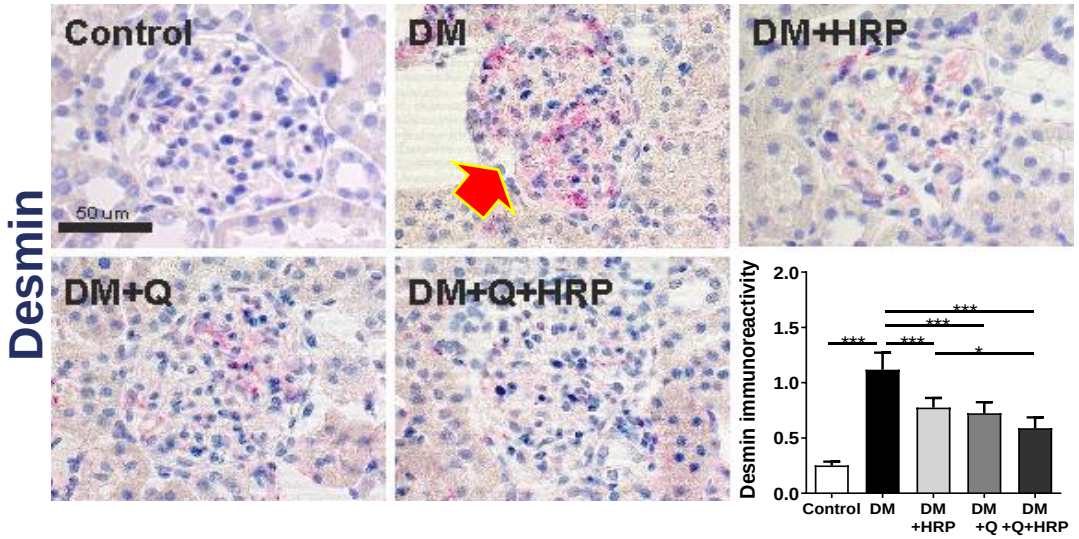
or

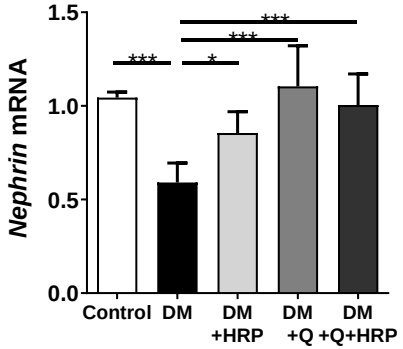
Combined Th. (Q+HRP)

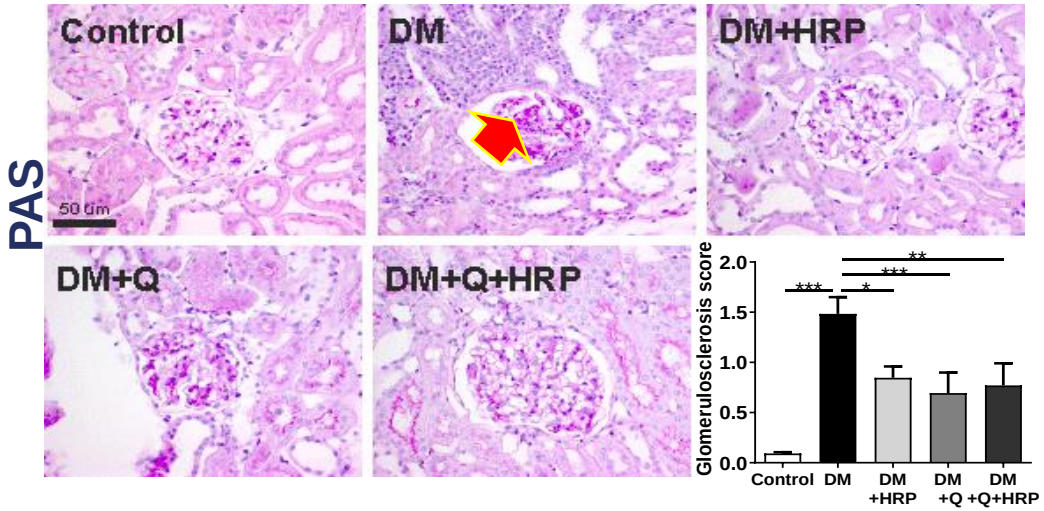


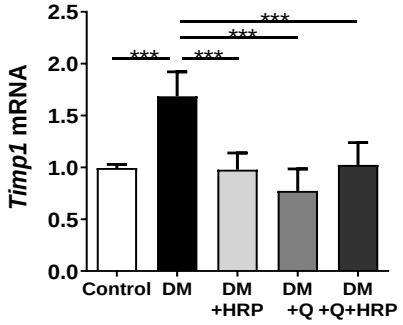


(P)RR and ACEi equally protect podocytes









In the rat model of T1DM both (pro)renin receptor- and ACE-inhibitor treatments reduce podocyte damage and kidney fibrosis with similar efficiency.

Kidney Blood Press Res 2017;42:109

PPARγ agonists:
a new hope to fight kidney fibrosis?

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International Nephrology Research and Training Center

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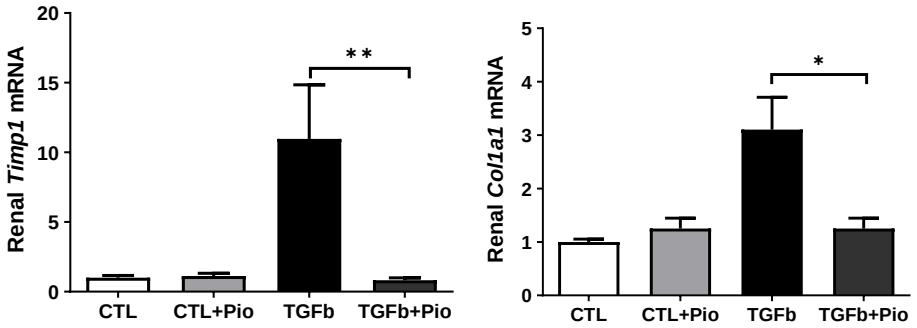
PPARγ agonists in kidney disease

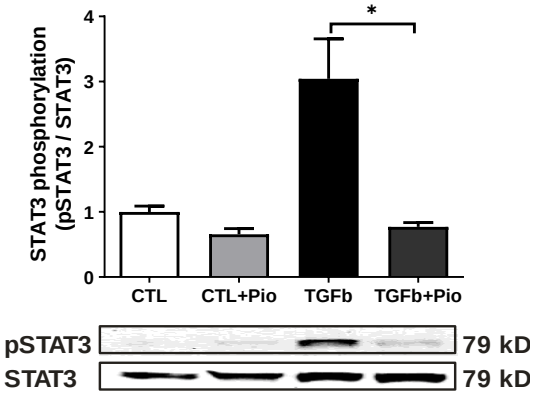
- PPARγ agonist treatment (**pioglitazone**, rosiglitazone):
  - Enhances insulin sensitivity, reduces gluconeogenesis and albuminuria in T2DM patients
    (Horm Metab Res. 2012; 44:914-918)
  - Reduces TGF-β production and kidney fibrosis in murine UUO model
    (Lab Invest. 2009; 89:47-58.)
  - Pioglitazon inhibits TGF-β signaling in VSMCs
    (Cell Metab, 2017, 25(5):1118-1134.e7)

- Possible effects of PPARγ on renal
  - STAT3-TGFβ-TIMP-1 axis
  - miRNA dysregulation
  - and autophagy were unknown.

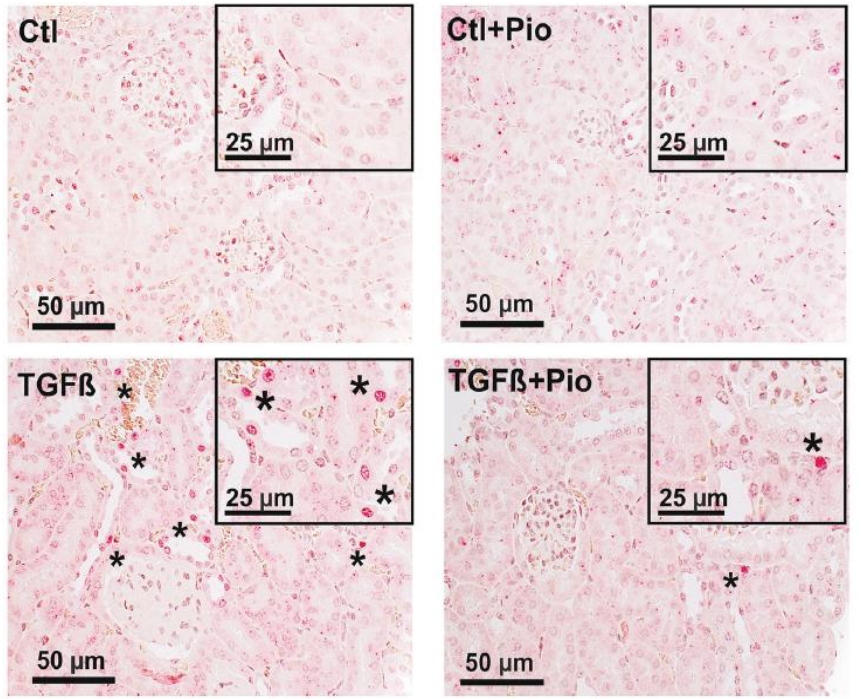
Pioglitazone counteracts TGF-β induced fibrotic efffects in mice

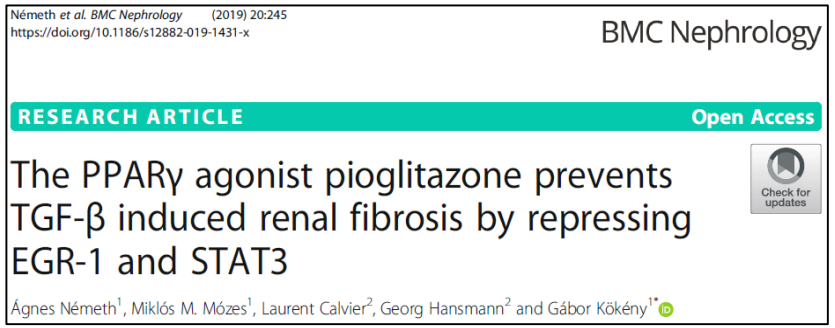
- TGFβ transgenic mouse model (elevated plasma TGF-β1, early fibrosis)
- Oral pioglitazone treatment for 5 weeks





Renal EGR1 expression



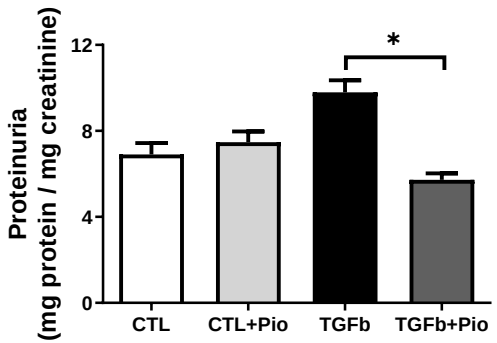

Németh et al. BMC Nephrology (2019) 20:245
https://doi.org/10.1186/s12882-019-1431-x

BMC Nephrology

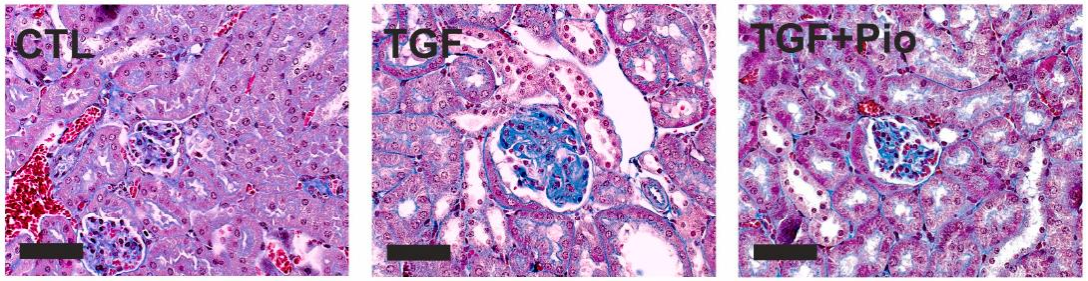
RESEARCH ARTICLE Open Access

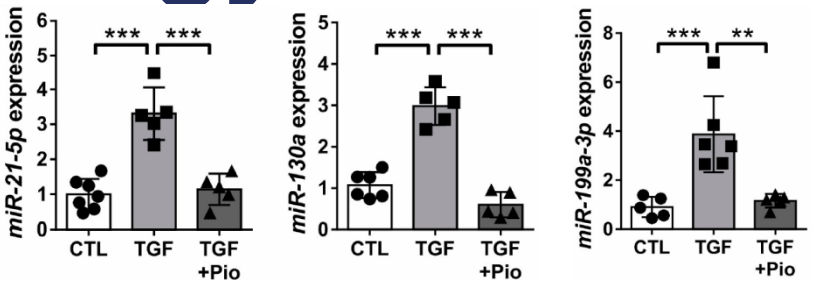
The PPARγ agonist pioglitazone prevents TGF-β induced renal fibrosis by repressing EGR-1 and STAT3

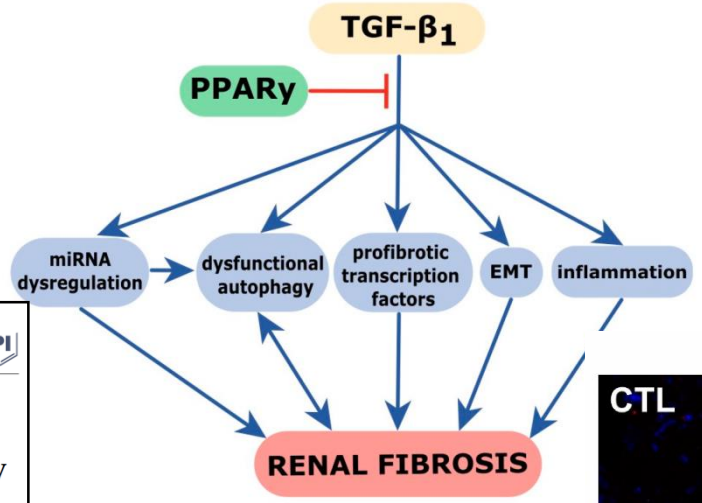
Ágnes Németh[1], Miklós M. Mózes[1], Laurent Calvier[2], Georg Hansmann[2] and Gábor Kökény[1*]

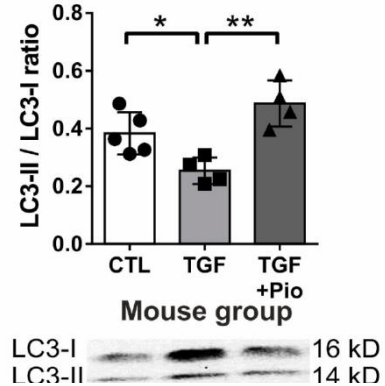


Pioglitazone reduced fibrotic miRNAs and restored autophagy in the kidneys









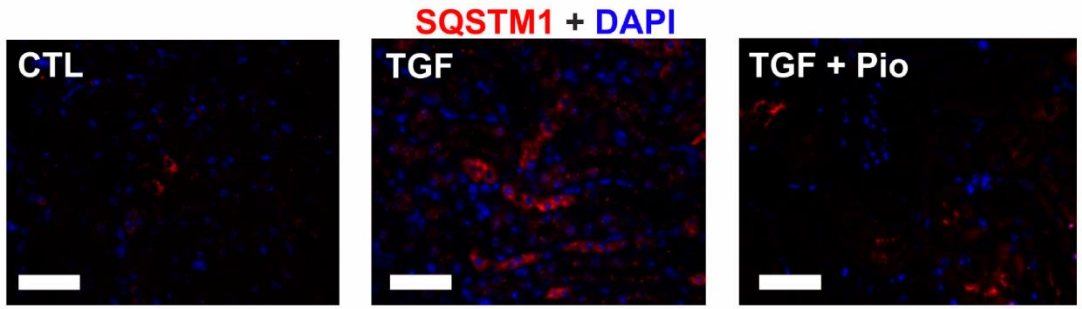
International Journal of Molecular Sciences
MDPI

Article

Pioglitazone Protects Tubular Epithelial Cells during Kidney Fibrosis by Attenuating miRNA Dysregulation and Autophagy Dysfunction Induced by TGF-β

Anna Manzéger [1,2], Gantsetseg Garmaa [1], Miklós M. Mózes [1,2], Georg Hansmann [3] and Gábor Kökény [1,2,*]

Int. J. Mol. Sci. **2023**, *24*, 15520. https://doi.org/10.3390/ijms242115520



Osmotic stress and kidney fibrosis

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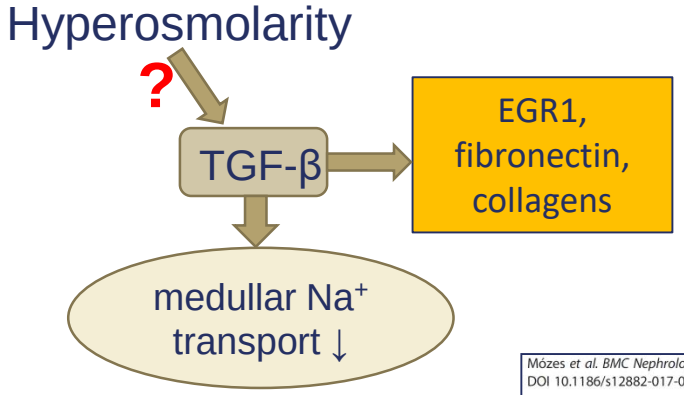
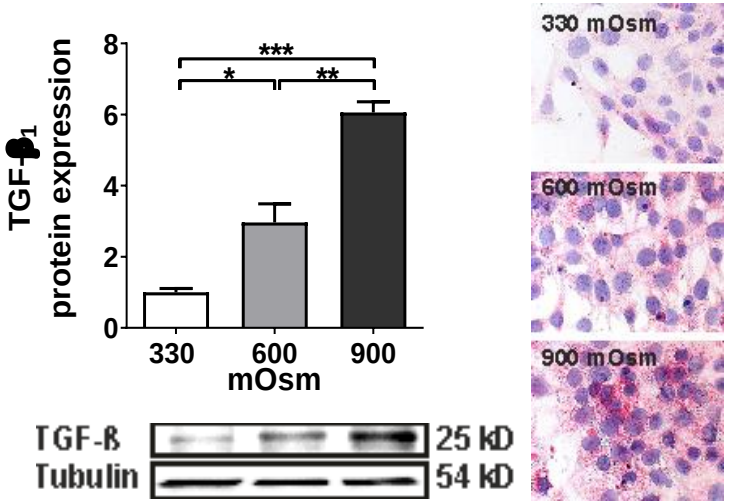
associate professor

Osmotic stress enhances TGF-β synthesis in the medullary collecting duct cells

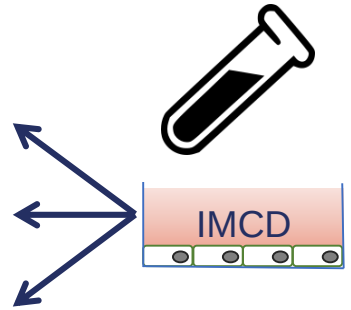


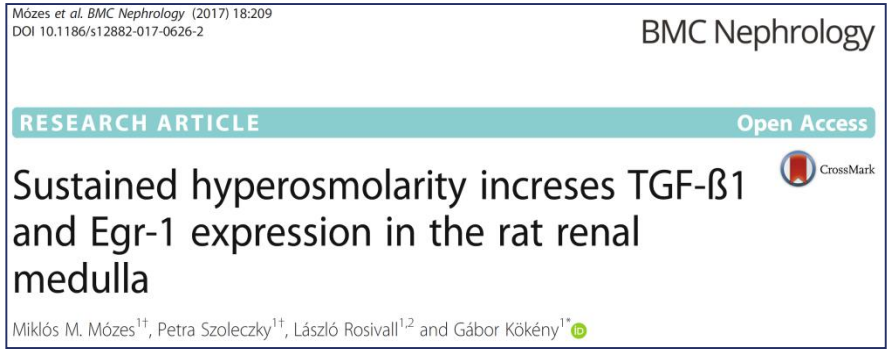


330 mOsm

600 mOsm

900 mOsm





Mózes *et al. BMC Nephrology* (2017) 18:209
DOI 10.1186/s12882-017-0626-2

BMC Nephrology

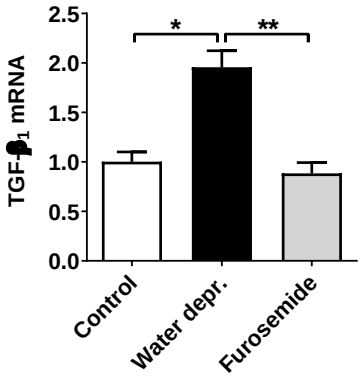
RESEARCH ARTICLE Open Access

Sustained hyperosmolarity increses TGF-ß1 and Egr-1 expression in the rat renal medulla

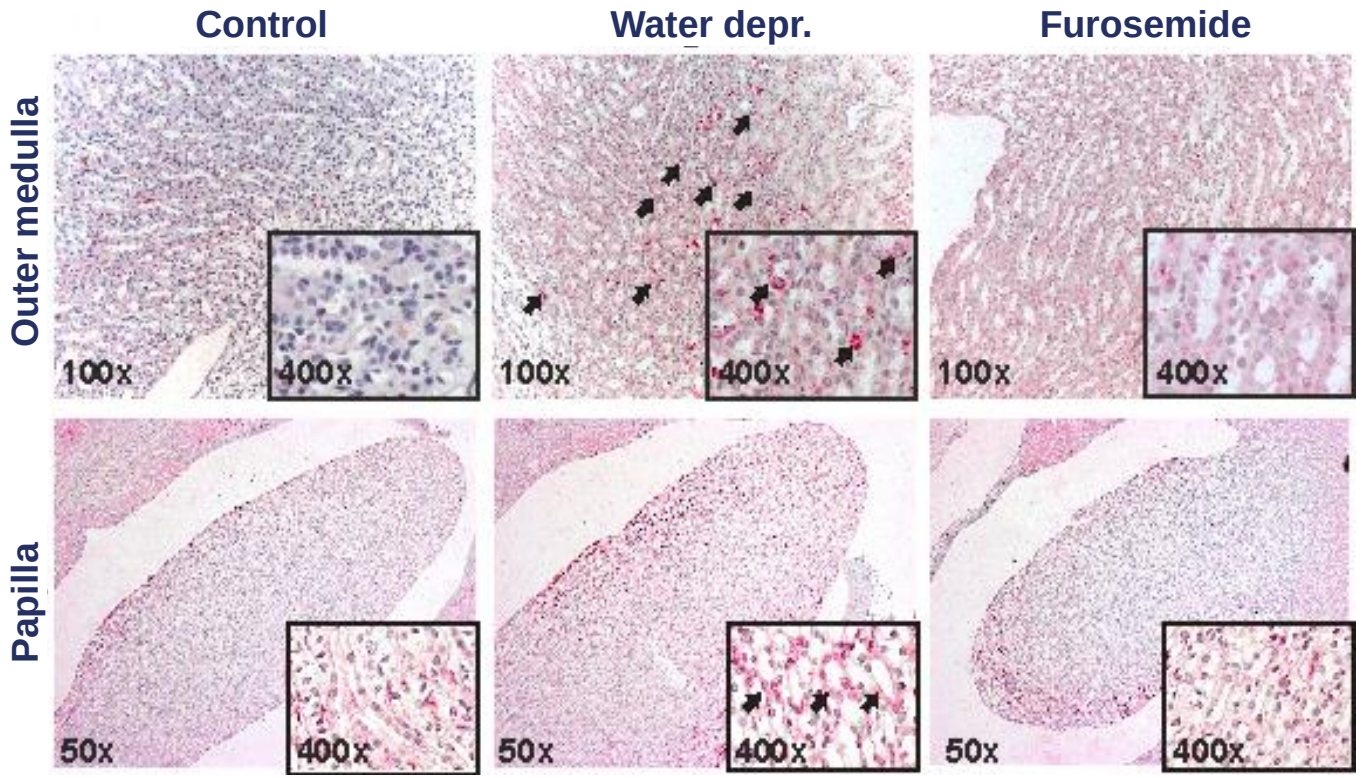
Miklós M. Mózes[1†], Petra Szoleczky[1†], László Rosivall[1,2] and Gábor Kökény[1*]

Osmotic stress enhances TGF-β synthesis in the renal medulla

5 days
- Control
- Water deprivation
- Diuretic treatment (Furosemide)

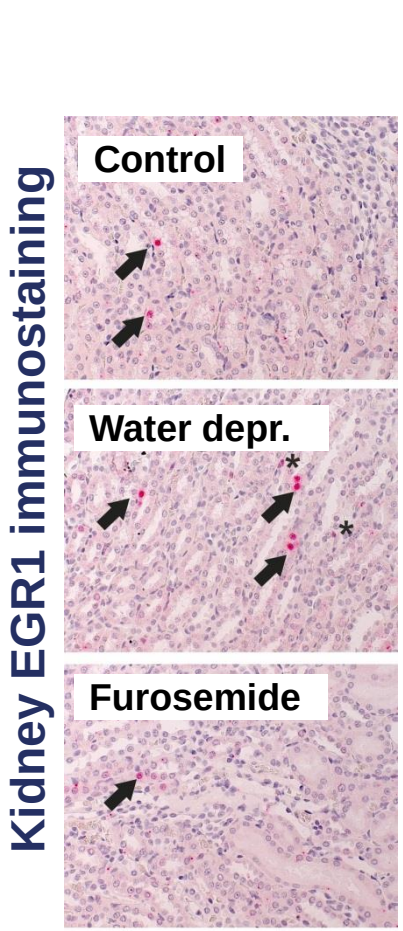


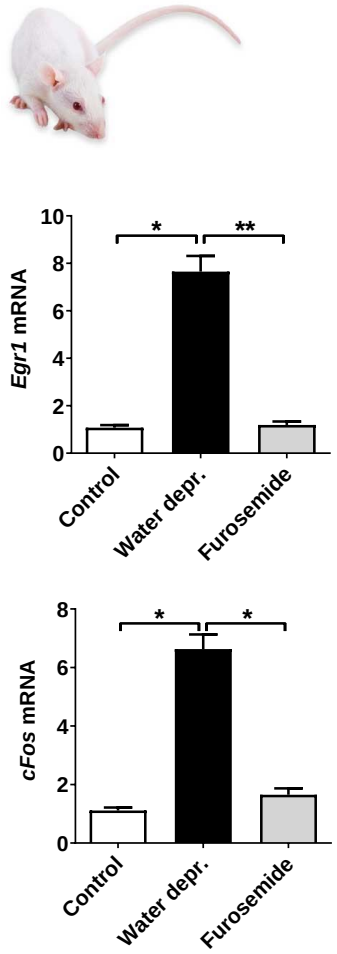
TGF-β immunostaining

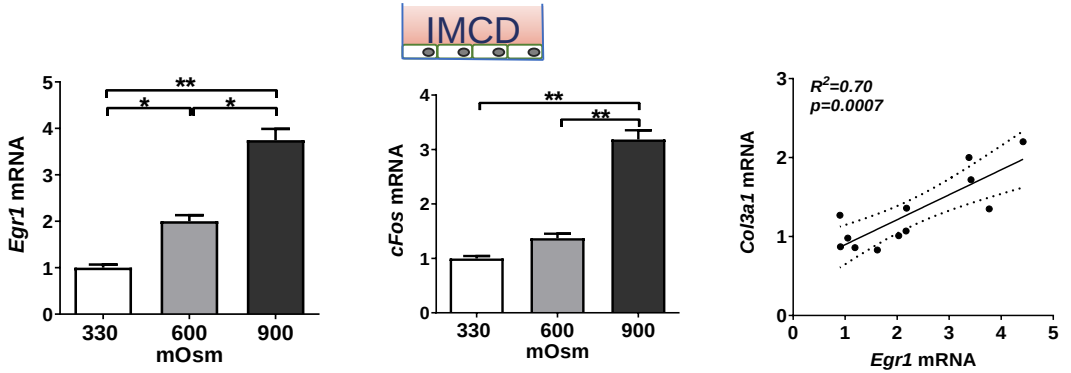


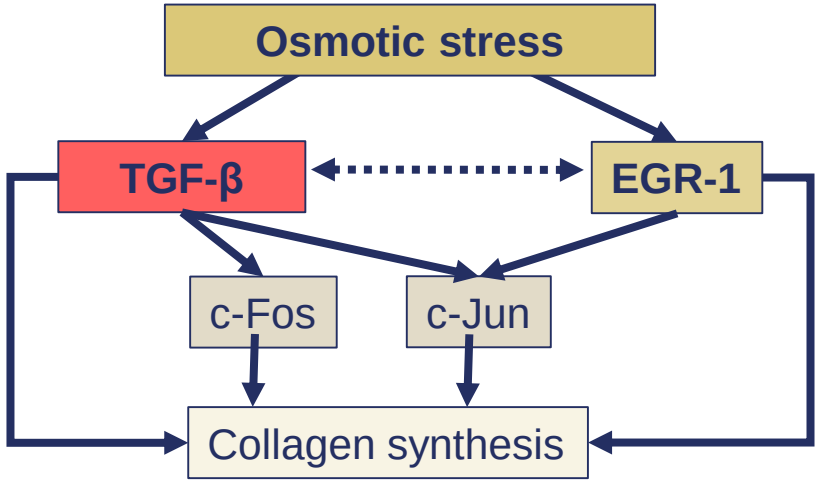
BMC Nephrol. 2017 Jul 3;18(1):209.

Osmotic stress induced pro-fibrotic pathways









Hyperosmolar environment in the renal medulla favors the activation of pro-fibrotic signaling pathways.

BMC Nephrol. 2017 Jul 3;18(1):209.

Do genetic factors determine kidney fibrosis progression?

Dr. habil. Gábor Kökény,
associate professor

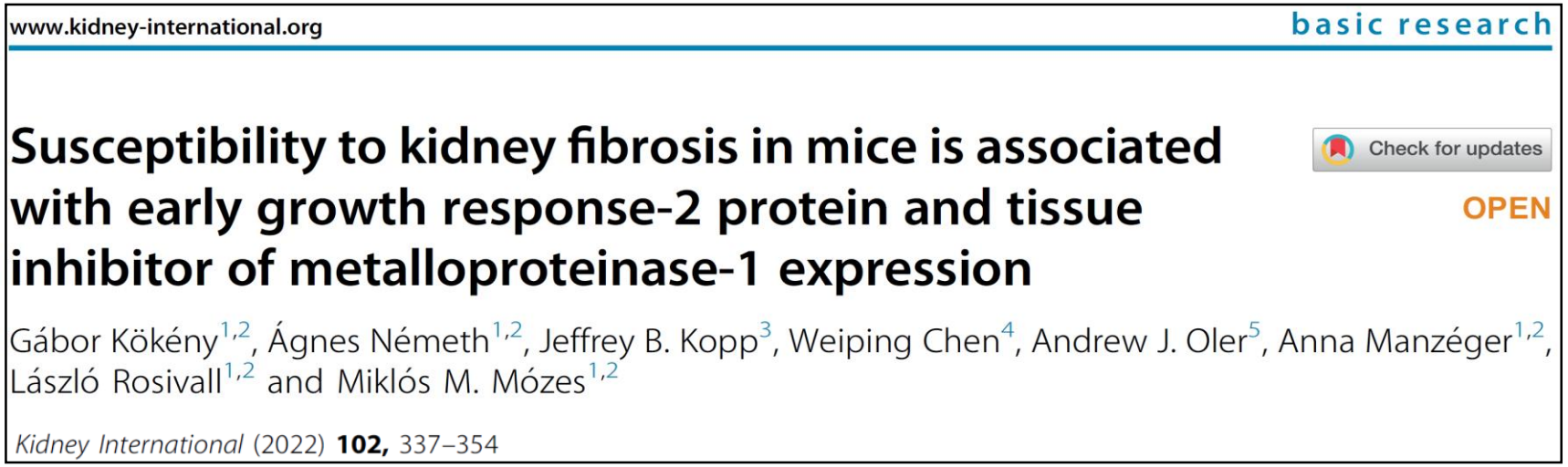
The potential role of genetic factors

- In mice, genetic background influences the progression of kidney diseases: BalbC is sensitive, C57Bl6 (B6) resistant.
  - ***It is not known whether there is a relationship between genetic background and renal TIMP-1 production.***

- EGR2 transcription factor is involved in scleroderma, but its role in renal fibrosis is not yet known.
  - ***The possible interaction between EGR2 and TIMP-1 is also unknown.***

Genetic background is a major determinant of renal fibrosis progression in mice

- TGFβ transgenic mice on different genetic backgrounds
- Early effects of unilateral ureteral obstruction (UUO) in B6 and CBA mice
- Study of human FSGS kidney biopsies



www.kidney-international.org basic research

Susceptibility to kidney fibrosis in mice is associated with early growth response-2 protein and tissue inhibitor of metalloproteinase-1 expression

Check for updates

OPEN

Gábor Kökény[1,2], Ágnes Németh[1,2], Jeffrey B. Kopp[3], Weiping Chen[4], Andrew J. Oler[5], Anna Manzéger[1,2], László Rosivall[1,2] and Miklós M. Mózes[1,2]

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Circulating TGF-β-induced fibrotic effect depends on genetic background in mice

B6

Collagen IV	Fibronectin	TGF-β1
B6-TGFβ	B6-TGFβ	B6-TGFβ
CBAxB6-TGFβ F1	CBAxB6-TGFβ F1	CBAxB6-TGFβ F1

CBA

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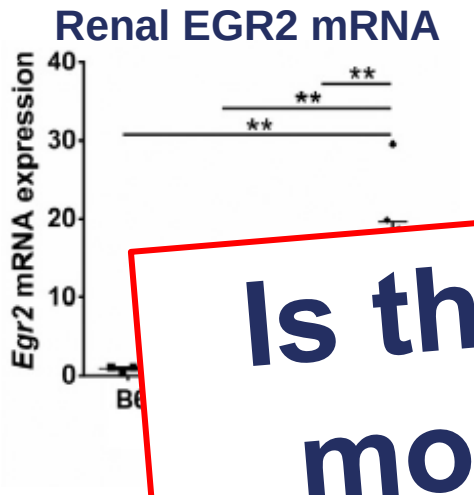
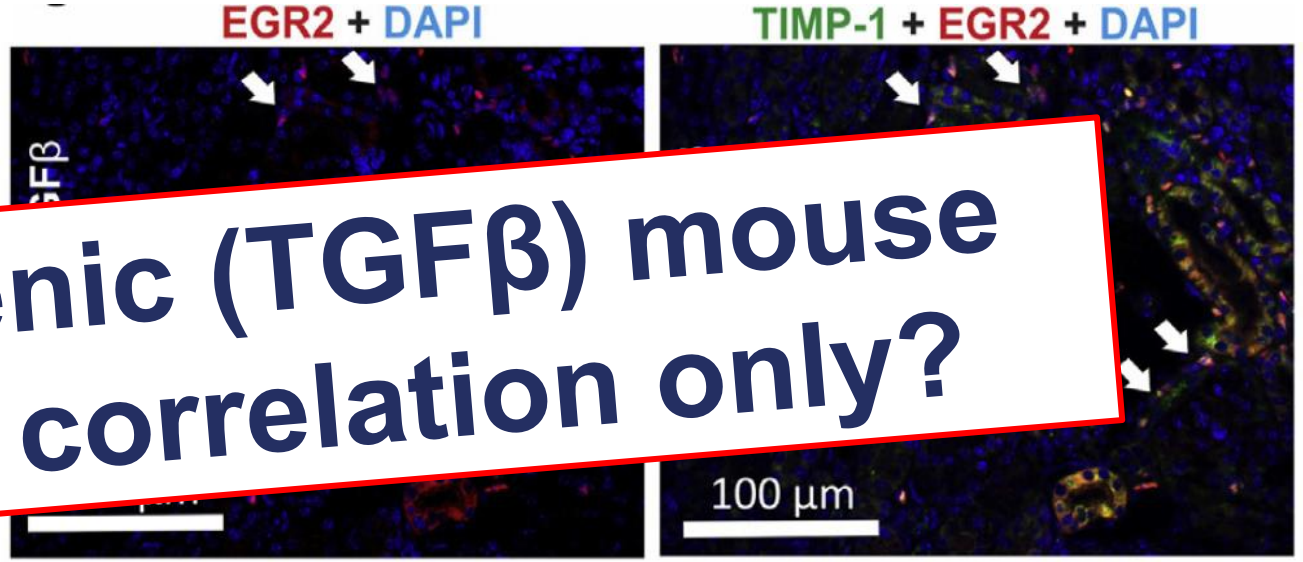
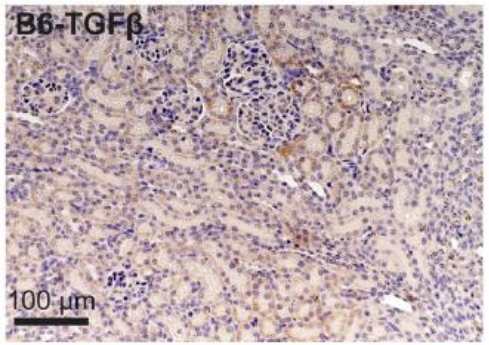
Genetic background determines TGF-β-induced renal EGR2 and TIMP-1 expression in mice

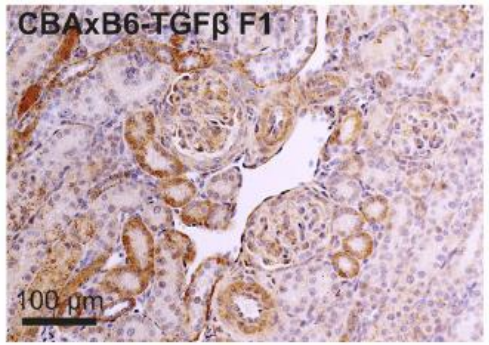




Is this a transgenic (TGFβ) mouse model specific correlation only?

Renal TIMP-1 protein





TIMP-1 and EGR2 highly co-localized in transgenic mouse kidneys.

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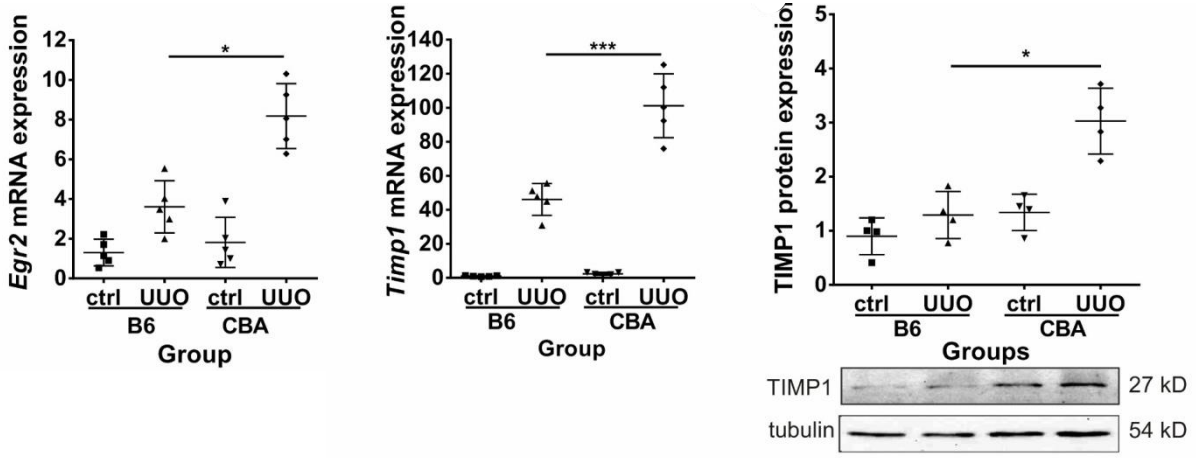
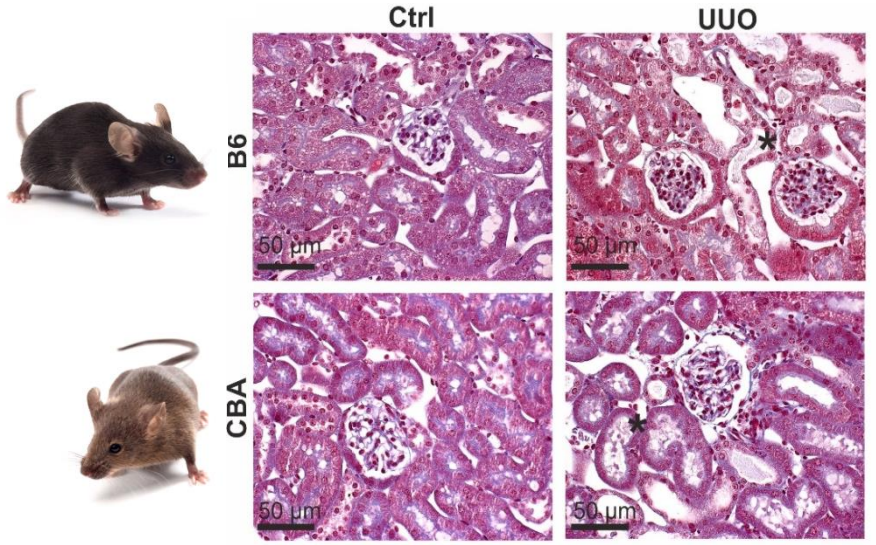
No histological difference, but very early EGR2 and TIMP1 induction after UUO

- UUO model:
  - Tubular dilatation (6h),
  - Tubular epithelial damage (24h),
  - Tubulointerstitial fibrosis (3-5 days),
  - Retrograde glomerular damage (7-14 days).



C57Bl6/J (B6) vs CBA
24h post-UUO



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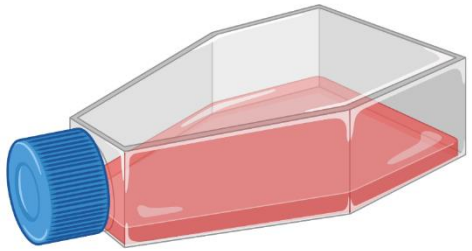
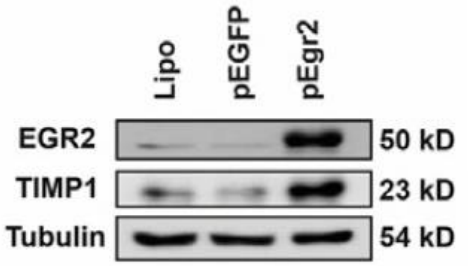
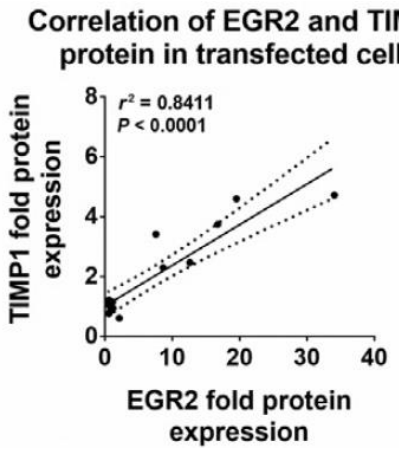
Is there a causal relationship between EGR2 and TIMP-1 *in vitro*?

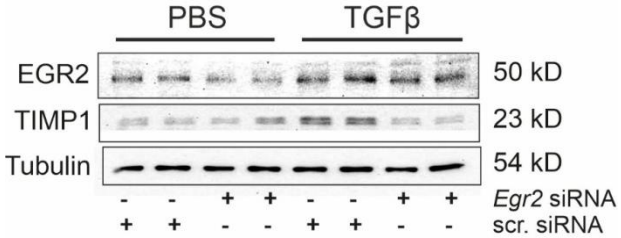
Tranfection of 293T cells with pCMV-*Egr2* construct

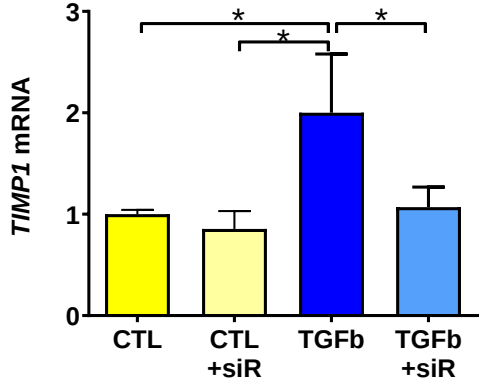






Silencing EGR2 in TGF-β treated HK2 cells





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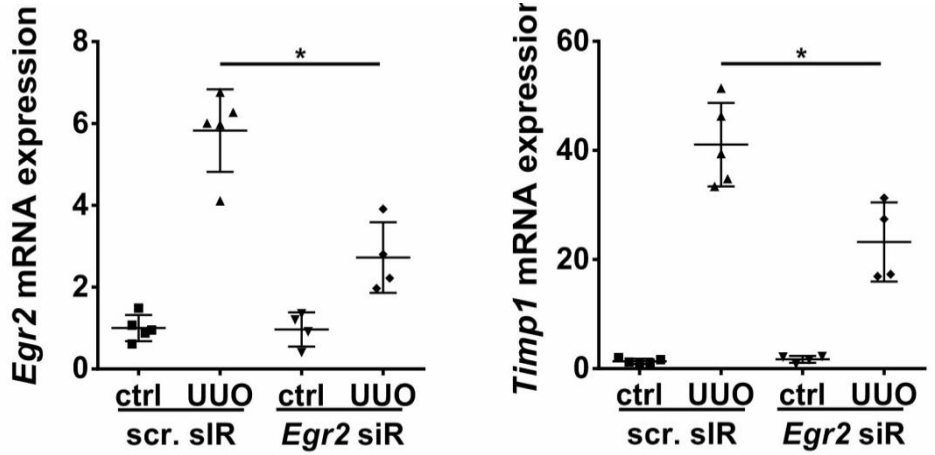
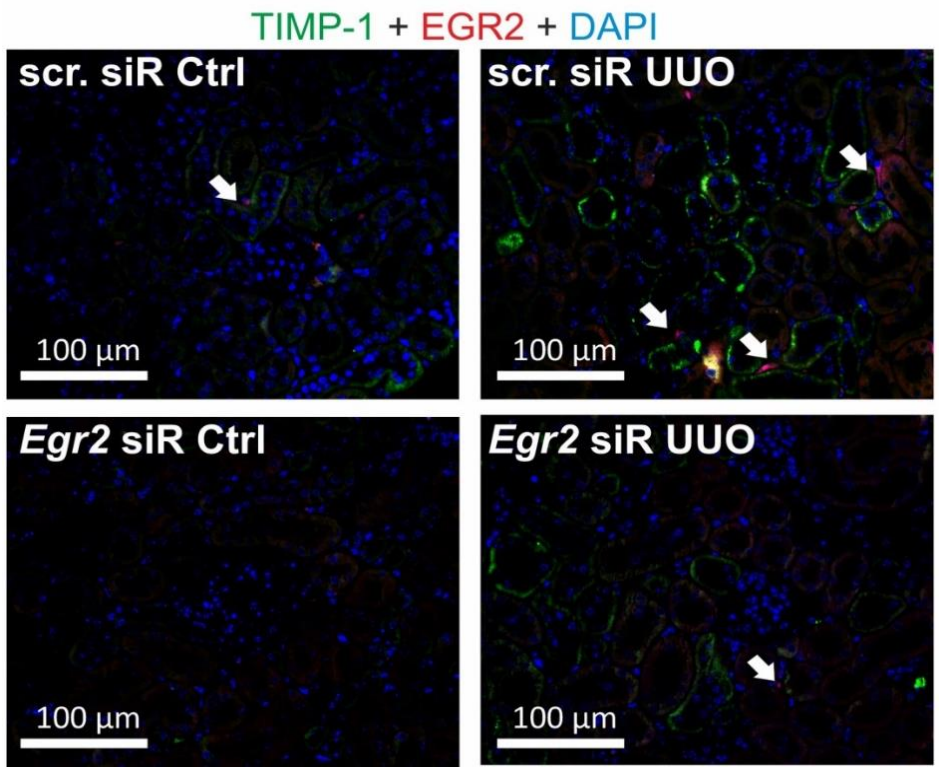
EGR2 silencing *in vivo* can suppress the production of renal TIMP1





* p<0.05; ** p<0.01; *** p<0.001

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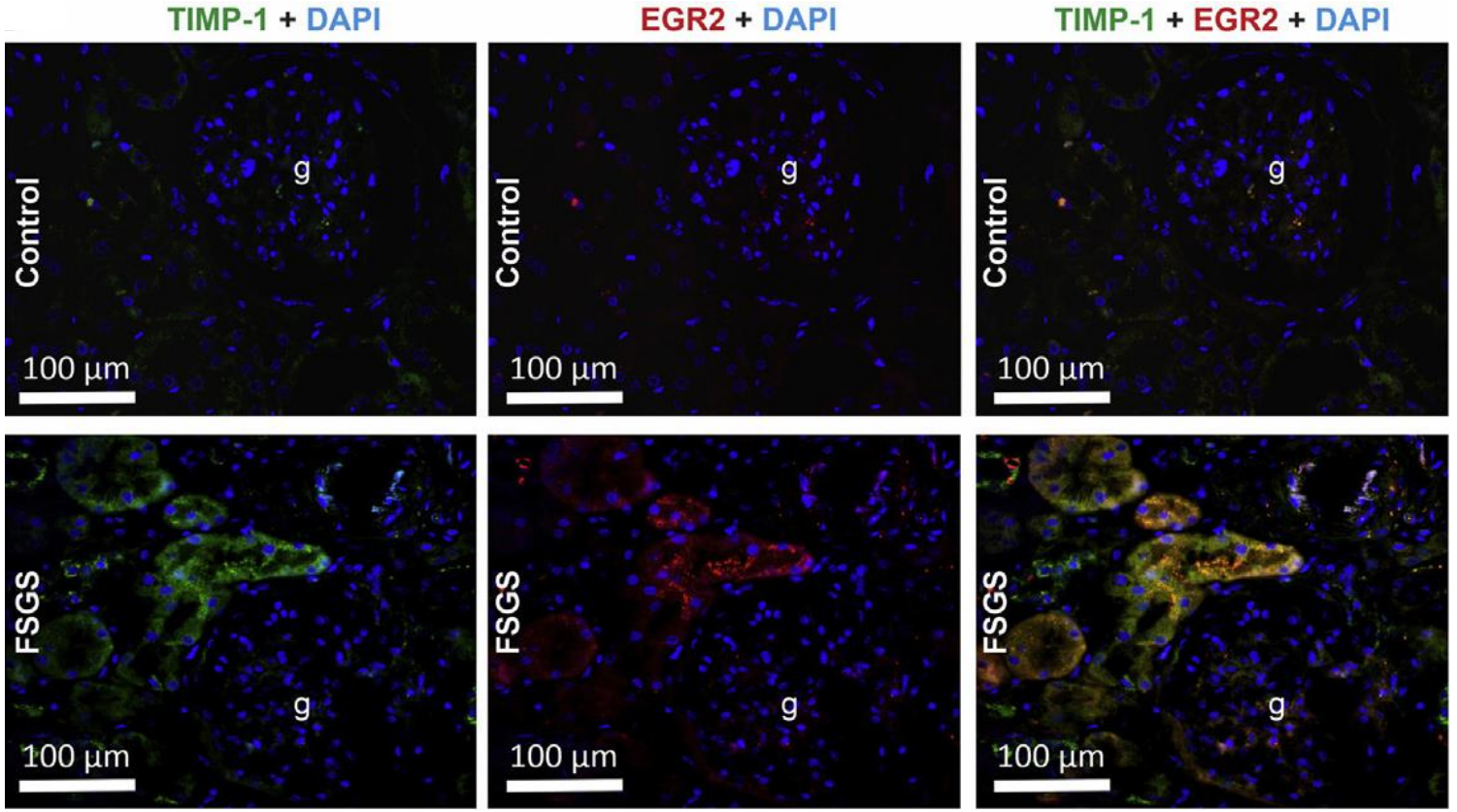
EGR2 and TIMP-1 are also over-expressed in fibrotic human kidneys (FSGS)



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Conclusions - Take home message

- Agents acting on the **NO-cGMP axis** may be a valuable add-on to classical therapies due to their **podocyte protecting and antifibrotic effects**.
- The PPARγ agonist **pioglitazone** may also have a role in the treatment of non-diabetic renal patients due to its potent anti-fibrotic effect.
- **Genetic background** by renal **EGR2** influences **the progression of renal fibrosis -** inhibiting EGR2 can slow the progression.

Thank you for your attention

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