TWEAK transactivates Epidermal growth factor receptor (EGFR) to modulate renal inflammation in the kidney.

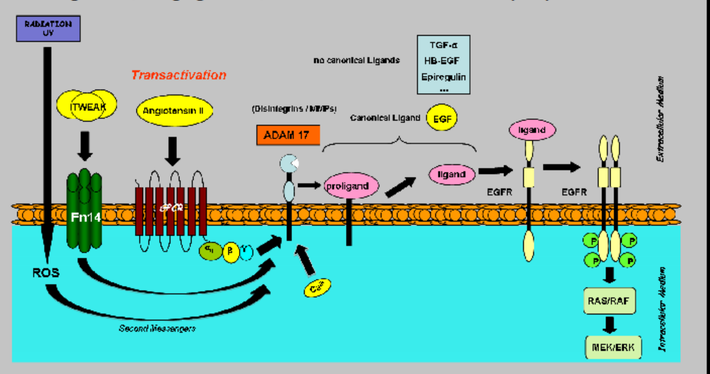
Sandra Rayego-Mateos [1], Jose Luis Morgado [1], Ana Sanz [4]; Satoru Eguchi [5];Janos Pato [2], Gyorgy Keri [2], J. Egido [3], A. Ortiz [4], M. Ruiz-Ortega [1].

1 Cellular Biology in Renal Diseases Laboratory. Universidad Autónoma Madrid. Spain. **2** Vichem Chemie Ltd. Budapest, Hungary and Semmelweis University,. **3** Renal Research Laboratory. Fundación Jiménez Díaz. Universidad Autónoma Madrid. Spain.**4** Dialysis Unit. Fundación Jiménez Díaz. Madrid Spain.**5** Temple University, Philadelphia,

INTRODUCTION

The cytokine tumor necrosis factor-like weak inducer of apoptosis (TWEAK) was described as a member of the tumor necrosis factor (TNF) superfamiy. TWEAK is produced as a cell surface-associated type II transmembrane protein, that after furin processing, the active form of TWEAK is released. TWEAK binds to Fn14 (fibroblast growth factor-inducible 14) with physiological affinity and exerts multiple biological activities, including stimulation of cell growth, angiogenesis, induction of inflammatory cytokines and apoptosis.

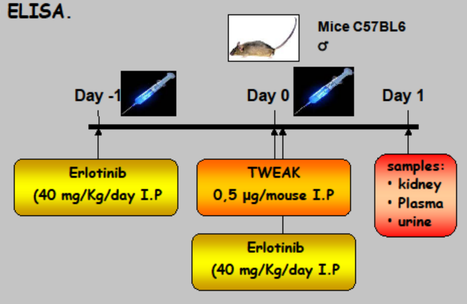
The Epidermal Growth Factor receptor (EGFR) transactivation has been involved in experimental renal fibrosis. EGFR transactivation is regulated by ADAMs (a disintegrin and metalloproteinase family), of which ADAM-17 is the best characterized member (Doedens 2003). TNF-α induces EGFR transactivation in a variety of cells (Yamaoka 2008; Argast 2004;Lee 2007), but there is no data about the potential relation between TWEAK and EGFR signalling.



METHODS

In vivo studies were performed in adult female C57Bl/6 mice (9-12 weeks old, 20 g). Systemic administration of TWEAK was done by a single intraperitoneal injection of 0,5 µg TWEAK/mouse. To block EGFR activation, animals were treated with the EGFR kinase inhibitor Erlotinib (40mg/Kg of body weight) or its vehicle (control group) 24 hours before TWEAK injection.

In vitro experiments were done in human and murine tubular epithelial cells, by different techniques: RT-PCR, Western blot or ELISA.



REFERENCES

1-Doedens, J. R., Mahimkar, R. M., and Black, R. A. **TACE/ADAM-17 enzymatic activity is increased in response to cellular stimulation.** Biochem Biophys Res Commun.2003;30331-338 .

2-Yamaoka, T., Yan, F., Cao, H., Hobbs, S. S., Dise, R. S., Tong, W., and Polk, D. B. **Transactivation of EGF receptor and ErbB2 protects intestinal epithelial cells from TNF-induced apoptosis.** Proc Natl Acad Sci U S A. 2008; 105, 11772-11777 .

3-Argast G.M., Campbell J.S., Brooling J.T., Fausto N.. **Epidermal growth factor receptor transactivation mediates tumor necrosis factor-induced hepatocyte replication,** J. Biol. Chem. 2004. 279;3453. .

4- Lee CW, Lin CC, Lin WN, Liang KC, Luo SF, Wu CB, Wang SW, Yang CM. **TNF-alpha induces MMP-9 expression via activation of Src/EGFR, PDGFR/PI3K/Akt cascade and promotion of NF-kappaB/p300 binding in human tracheal smooth muscle cells.** Am J Physiol Lung Cell Mol Physiol. 2007/9981.

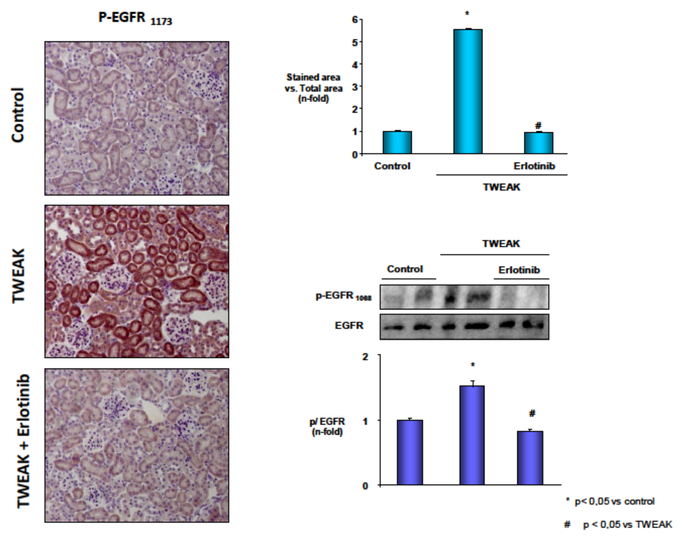
AIM

To study whether EGFR transactivation is involved in TWEAK-mediated renal responses.

RESULTS

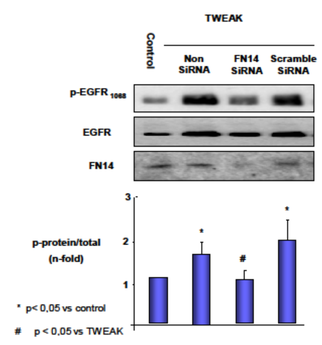
Tweak induces EGFR phosphorylation in the kidney

Intraperitoneal administration of recombinant TWEAK into mice increased renal phosphorylated EGFR protein levels (p-EGFR), compared to control mice. The EGFR kinase inhibitor Erlotinib diminished renal p-EGFR level to control values.



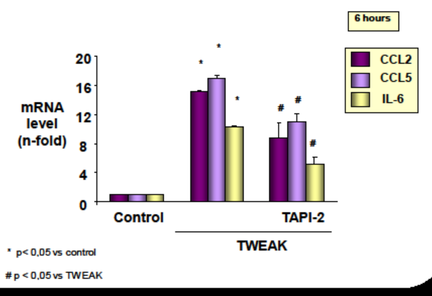
TWEAK thougth its receptor, Fn14 induces EGFR transactivation

In tubular epithelial cells, gene silencing of Fn14, blocked TWEAK-induced EGFR transactivation.



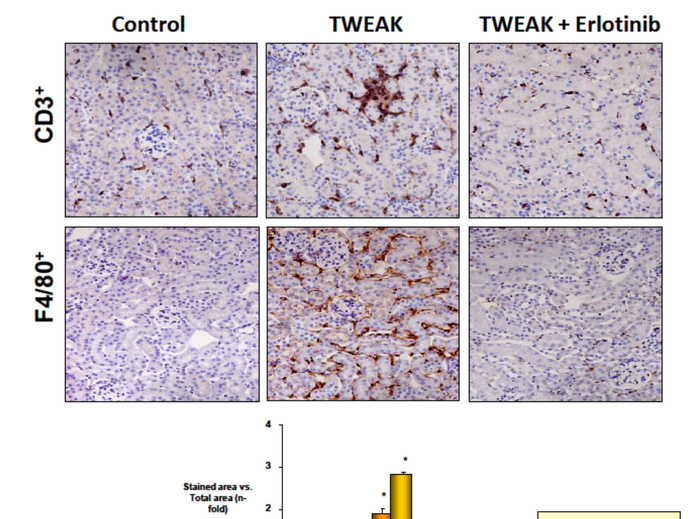
TWEAK regulates proinflammatory mediators via ADAM17 in vitro

In cultured murine tubular epithelial cells, preincubation with ADAM17 inhibitor prevented TWEAK-induced gene upregulation of the proinflammatory mediators CCL2 , CCL5 and IL-6.



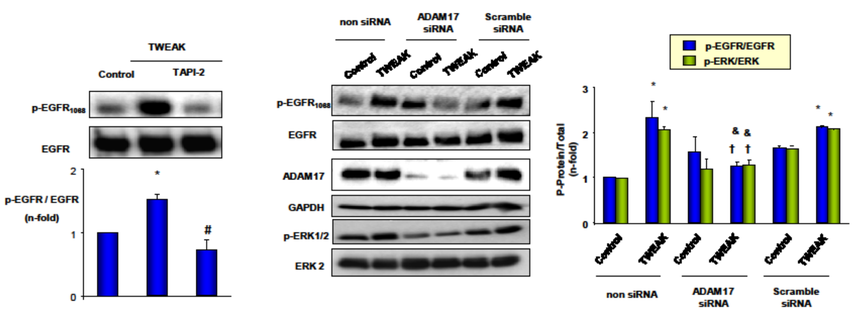
The EGFR kinase inhibitor erlotinib diminishes TWEAK-induced renal damage

TWEAK induced the presence of infiltrating monocytes/macrophages (F4/80+) and T cells (CD3+) in renal interstitium, that was markedly diminished by Erlotinib treatment. Moreover, down-regulation of renal production of proinflammatory factors (CCL2, IL-6, CCL5 and CCR2) was also observed in Erlotinib-treated TWEAK-injected mice.



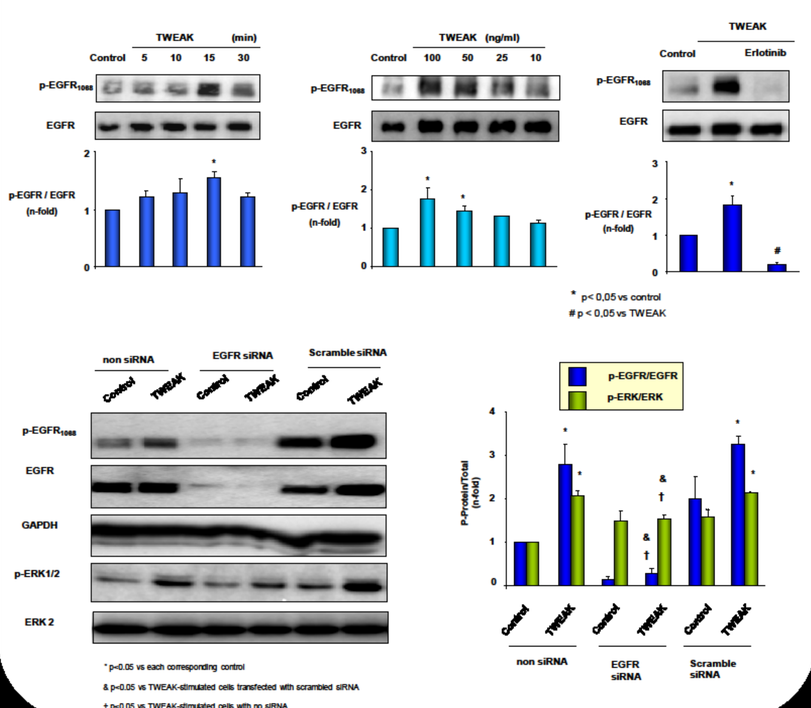
TWEAK induces EGFR transactivation via ADAM17

In tubular epithelial cells, the pharmacological inhibition of ADAM-17, using TAPI-2, or the gene silencing of ADAM-17, significantly inhibited TWEAK-induced EGFR phosphorylation.



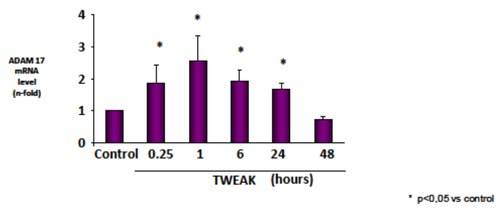
TWEAK induces EGFR phosphorylation and ERK activation in cultured tubular epithelial cells

In cultured murine tubular epithelial cells, TWEAK increased EGFR phosphorylation levels, as early as 15 minutes, that was inhibited by pharmacological EGFR kinase inhibition (Erlotinib) or EGFR gene silencing.



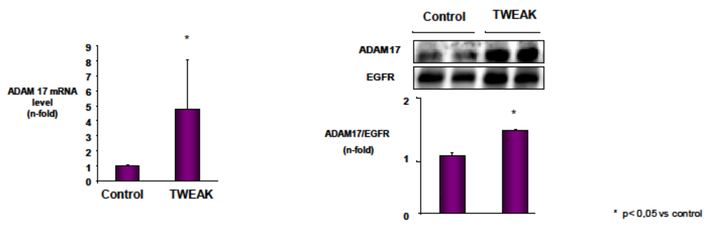
TWEAK induces ADAM17 activation *in vitro*

In cultured human tubular epithelial cells, TWEAK induced ADAM17 upregulation, as early as 15 minutes, that was observed until 24 hours.



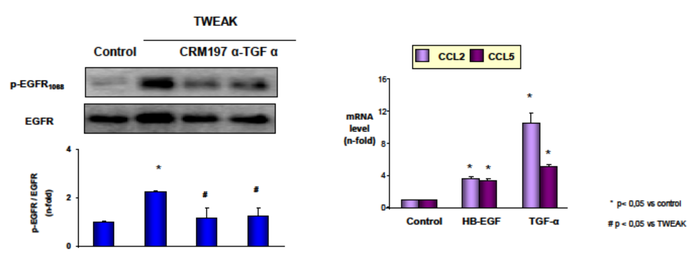
TWEAK induces ADAM17 activation in the kidney

In the kidney of TWEAK inyected mice, we found that ADAM17 was upregulated, at gene and protein levels, observed by real-time PCR and Western Blot .



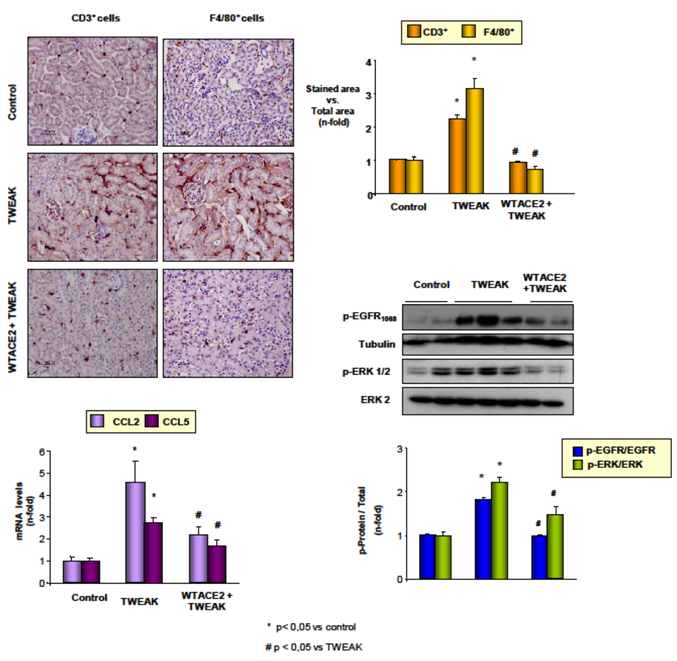
TWEAK induces the release of HB-EGF and TGF-α ligands *in vitro*

In HK2 cells, the pharmacological inhibition of HB-EGF, by CRM197, diminished EGFR phosphorylation caused by TWEAK. TGF-α blockade, using a specific neutralizing antibody, inhibited TWEAK-mediated EGFR activation. Moreover, the stimulation of MCT cells with the recombinant ligands HB-EGF or TGF-α increased proinflammatory gene expression.



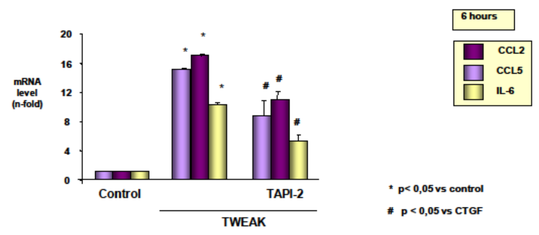
The ADAM-17 inhibitor WTACE2 diminished TWEAK-induced proinflammatory responses in the kidney

ADAM-17 inhibition diminished renal p-EGFR levels in TWEAK-treated mice to values similar to control mice. Pretreatment with WTACE2 diminished the renal proinflammatory response caused by TWEAK, decreasing the number of infiltrating inflammatory cells and significantly downregulated proinflammatory gene expression (CCL2 and CCL5).



TWEAK regulates proinflammatory mediators via EGFR activation *in vitro*

In cultured murine tubular epithelial cells, preincubation with erlotinib prevented TWEAK-induced gene upregulation of the proinflammatory mediators CCL2, CCL5 and IL-6.



CONCLUSIONS

-TWEAK induces EGFR phosphorylation in the kidney, mainly in tubular epithelial cells.

-The EGFR kinase inhibitor Erlotinib diminishes the renal inflammatory response caused by TWEAK.

- TWEAK transactivates EGFR through Fn14 binding and activation of ADAM17.

-EGFR transactivation induced by TWEAK is linked to ERK activation and upregulation of proinflammatory genes.

These results suggest that EGFR transactivation is an important mechamisn involved in TWEAK-induced renal inflammatory response. Blocking EGFR transactivation could be a novel therapeutic target for inflammatory renal pathologies.

50ERA-EDTA

359--SP

Cell signalling

Sandra Rayego Mateos

DOI: 10.3252/pso.eu.50era.2013

Poster presented at: ERA-EDTA 50th Congress Istanbul, Turkey May 15-21, 2013

PosterSessionOnline.com