The inflammatory cytokine TWEAK decreases PGC-1α expression and mitochondrial function in acute kidney injury

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Studies of mitochondria-targeted nephroprotective agents suggest a key role of mitochondrial injury in AKI. Here we tested whether an improved perception of factors responsible for mitochondrial biogenesis may provide clues to novel therapeutic approaches to AKI. TWEAK is an inflammatory cytokine which is upregulated in AKI. Transcriptomic analysis of TWEAK-stimulated cultured murine tubular epithelial cells and folic acid-induced AKI in mice identified downregulation of peroxisome proliferator-activated receptor-γ coactivador-1α (PGC-1α) and its target genes (mitochondrial proteins Ndufs1, Sdha, and Tfam) as a shared feature. Neutralizing anti-TWEAK antibodies prevented the decrease in kidney PGC-1α and its targets during AKI. TWEAK stimulation decreased kidney PGC-1α expression in healthy mice and decreased expression of PGC-1α and its targets as well as mitochondrial membrane potential in cultured tubular cells. Adenoviral-mediated PGC-1α overexpression prevented TWEAK-induced downregulation of PGC-1α-dependent genes and the decrease in mitochondrial membrane potential. TWEAK promoted histone H3 deacetylation at the murine PGC-1α promoter. TWEAK-induced downregulation of PGC-1α was prevented by histone deacetylase or NF-κB inhibitors. Thus, TWEAK decreases PGC-1α and target gene expression in tubular cells *in vivo* and *in vitro*. Approaches that preserve mitochondrial function during kidney injury may be therapeutic for AKI.

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Acute kidney injury (AKI) is a syndrome characterized by an acute, usually transient decrease in the kidney function resulting from kidney damage.[1] AKI is associated with high mortality and accelerated progression of chronic kidney disease.[2] The incidence of AKI is increasing as a consequence of a more fragile aging population and the existence of a consensus definition of AKI.[2] Currently, there is no satisfactory therapy to prevent progression or accelerate recovery from AKI.[1,3] In recent years, functional interventional evidence has emerged of a key role of mitochondrial biology in AKI.[4] Several mitochondria-targeted nephroprotective agents have been successfully tested in experimental AKI, and clinical trials are ongoing.[4] An improved understanding of the molecular regulation of mitochondrial biogenesis may provide clues to design novel therapeutic approaches to kidney injury.

PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) is a member of a small family of transcriptional regulators that controls the expression of genes involved in energy homeostasis, mitochondrial biogenesis, fatty acid oxidation, and glucose metabolism. PGC-1α is pivotal for the mitochondrial function, as well as for the expression of key mitochondrial proteins.[5] PGC-1α downregulation has been observed in different experimental models of AKI,[6,7] and it is implicated as a causative event in renal functional impairment during sepsis-associated AKI.[8] During sepsis kidney PGC-1α expression is suppressed in proportion to the degree of organ dysfunction and rises back to preinjury levels as the function normalizes.[8] However, sepsis is a specific form of AKI, in which dysfunction is disproportionate in relation to morphological histological injury. Thus, we have now explored experimental folic acid nephropathy, a model of renal injury, that shares with human AKI the development of extensive cell death, interstitial inflammatory infiltrates, and tubular cell proliferation that restores tubular cell mass.[9] In both folic acid–induced and ischemia–reperfusion AKI, functional interventional studies have disclosed a key role of TWEAK.[9,10] TWEAK is a tumor necrosis factor (TNF) superfamily cytokine that promotes kidney injury in the setting of AKI, immune-mediated glomerular injury, and nonimmune proteinuric kidney disease.[9,11,12] An ongoing clinical trial is exploring

nephroprotection by neutralizing anti-TWEAK antibodies in human lupus nephritis.[13] However, much remains to be understood of the molecular mechanisms of nephroprotection resulting from TWEAK neutralization, and unraveling these pathways may help design novel nephroprotective therapeutic strategies.

TNF superfamily members may contribute to tissue injury by activating the transcription factor nuclear factor (NF)-κB.[14] Unlike TNFα, the best-characterized member of the cytokine superfamily, TWEAK activates both canonical and noncanonical NF-κB signaling.[15,16] Thus, TWEAK functions may be distinct from those of TNFα.[15] It was recently reported that PGC-1α downregulation mediates the deleterious effects of TWEAK in cardiac disease and in skeletal muscle atrophy. Indeed, TWEAK decreased PGC-1α expression in cardiomyocytes and skeletal muscle.[17,18] In kidney cells, TWEAK activation of NF-κB not only leads to increased expression of inflammatory genes but also to decreased expression of nephroprotective genes such as Klotho.[19,20] As PGC-1α appears to have a role in renal protection, we hypothesized that TWEAK may also downregulate PGC-1α expression in kidney cells. We took advantage of transcriptomics studies of experimental AKI and of TWEAK-stimulated tubular cells to search for mitochondrial biogenesis regulatory genes that were coordinately regulated by AKI and by TWEAK. This transcriptomics approach may identify TWEAK targets that may be relevant for the nephroprotective effect of anti-TWEAK-neutralizing antibodies. We found that TWEAK decreased the expression of PGC-1α in tubular cells both in culture and *in vivo* and that TWEAK targeting preserved PGC-1α in AKI. Moreover, decreased PGC-1α expression was associated with decreased expression of PGC-1α-dependent genes encoding mitochondrial proteins. Both NF-κB activation and histone deacetylation contributed to decrease PGC-1α in response to TWEAK.

RESULTS

TWEAK downregulates PGC-1α in AKI

The mechanisms that regulate PGC-1α expression during renal injury were explored in an established mouse model of AKI induced by a folic acid overdose. In this model, renal injury and loss of renal function are associated with interstitial inflammation and tubular injury.[9] A kidney transcriptomic analysis showed reduced kidney PGC-1α mRNA in AKI at 24 h compared with healthy mice (AKI/healthy kidney ratio 0.623 ± 0.497; $P < 0.01$; false discovery rate 0.02).[21] Real time–PCR confirmed a significant decrease in PGC-1α mRNA levels at 24 and 72 h after injury (Figure 1a). There was a negative correlation between PGC-1α mRNA and blood urea nitrogen levels, a marker that increases with severity of AKI (Figure 1b). At day 7, PGC-1α mRNA levels had been restored (Figure 1a), similar to renal function (Supplementary Figure S1A online). As TWEAK modulates PGC-1α expression in nonrenal cells,[17] we explored the role of TWEAK in regulating PGC-1α expression in AKI. The TWEAK receptor Fn14 was significantly upregulated in AKI, where it localized to tubular cells (Figure 1c and Supplementary Figure S2 online) and Fn14 expression positively correlated with blood urea nitrogen (Figure 1d) and negatively correlated with PGC-1α expression (Figure 1e). We previously observed that neutralizing anti-TWEAK antibodies prevented renal dysfunction at 72 h in folic acid–induced AKI[9,12] (Supplementary Figure S1B online). We have now observed that neutralizing anti-TWEAK antibodies prevented the decrease in PGC-1α mRNA and protein expression 72 h after injury (Figure 1f–h). In contrast, neutralizing anti-TWEAK antibodies did not significantly improve early kidney dysfunction (Supplementary Figure S3A online) and did not prevent PGC-1α downregulation at 24 h after injury (Supplementary Figure S3B online). This result suggests that TWEAK may contribute to amplify and promote persistence of renal injury through PGC-1α downregulation.

Suppression of mitochondrial gene transcripts during AKI

The transcriptome of folic acid–induced AKI shows downregulation of PGC-1α-dependent gene transcripts encoding mitochondrial proteins (Table 1). In addition, the transcriptome of cultured murine renal tubular cells treated with 100 ng/ml TWEAK for 6 h also showed downregulation of PGC-1α mRNA (TWEAK/control ratio 0.583 ± 0.072; $^*P < 0.004$; false discovery rate 0.09) and PGC-1α targets genes (Table 1). Comparison of both transcriptomes identified three mitochondrial protein-encoding genes that were downstream of PGC-1α and significantly downregulated in both conditions, *Ndufs1, Sdha,* and *Tfam,* encoding NADH dehydrogenase (ubiquinone) Fe-S protein 1, succinate dehydrogenase complex subunit A flavoprotein (Fp), and mitochondrial transcription factor A, respectively (Table 1).[8,22] These genes were selected for further studies. We confirmed downregulation of PGC-1α, Ndufs1, Sdha, and Tfam mRNA during AKI by reverse transcriptase–PCR (Figure 2a) and observed a positive correlation of PGC-1α expression with the expression of these PGC-1α target genes during AKI (Figure 2b). Finally, neutralizing anti-TWEAK antibodies prevented the decreased mRNA expression of PGC-1α targets during AKI (Figure 2c). These results suggest that during AKI, downregulation of PGC-1α could lead to impairment of the mitochondrial function through downregulation of PGC-1α targets genes, and this is dependent on TWEAK.

Exogenous TWEAK decreases renal PGC-1α expression *in vivo*

TWEAK blockade prevented downregulation of renal PGC-1α expression in AKI, but this could be a direct effect or an indirect consequence of improved tissue injury. Thus, we explored whether TWEAK directly regulated kidney PGC-1α expression in healthy kidneys *in vivo.* Systemic injection of TWEAK decreased renal PGC-1α mRNA levels *in vivo* at 48 and 72 h (Figure 3a). Moreover, TWEAK also decreased PGC-1α protein levels *in vivo* as assessed by western blot (Figure 3b and c).

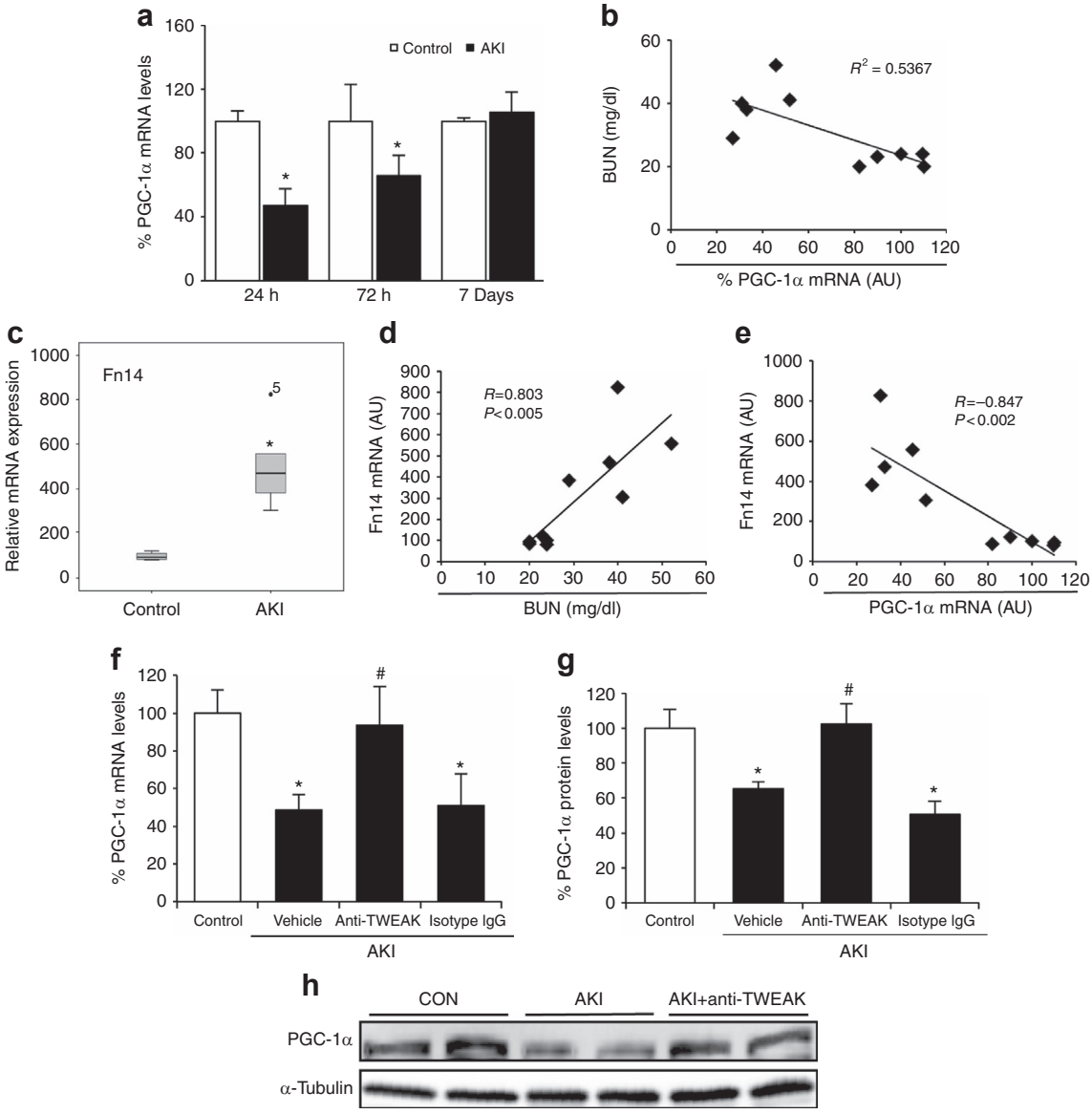

Figure 1 | TWEAK neutralization prevented PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) downregulation during acute kidney injury (AKI). (a) Kidney PGC-1α mRNA expression was assessed by real-time quantitative real time–PCR (qRT-PCR) in mice with AKI induced by a folic acid overdose or controls at different periods of time. Mean ± SEM of five animals per group. *$P < 0.02$ versus control. **(b)** Scatter plot showing the significant negative correlation between kidney mRNA expression of PGC-1α and serum blood urea nitrogen (BUN) levels in AKI at 72 h ($n = 10$). **(c, d)** Fn14 mRNA renal expression (RT-PCR) is upregulated in AKI at 72 h and positively correlates with serum BUN levels. Mean ± SEM of five animals per group. *$P < 0.01$ versus control. **(e)** Scatter plot showing the significant negative correlation between kidney mRNA expression for Fn14 and PGC-1α in AKI at 72 h ($n = 10$). **(f)** TWEAK neutralization with specific antibodies prevented PGC-1α mRNA downregulation in kidneys at 72 h after AKI induction. Mean ± SEM of 5–6 animals per group. *$P < 0.01$ versus control, #$P < 0.03$ versus AKI with/without immunoglobulin G (IgG) control. **(g)** Total kidney PGC-1α protein, measured by western blot, is also reduced in AKI at 72 h, and this reduction is prevented by TWEAK antagonism. Mean ± SEM of 5–6 animals per group. *$P < 0.01$ versus control, #$P < 0.03$ versus AKI. **(h)** Representative western blot of PGC-1α expression in lysates from whole kidneys.

TWEAK decreases PGC-1α expression in cultured renal tubular cells

Next, we studied the effect of TWEAK over cultured renal cells. TWEAK decreased PGC-1α mRNA expression in a dose- and time-dependent manner (Figure 4a and b). TWEAK also decreased PGC-1α protein levels in tubular cells (Figure 4c). These results suggest that TWEAK-induced PGC-1α downregulation in the kidney is a direct effect of the cytokine over renal tubular cells.

TWEAK negatively affects mitochondrial function through PGC-1α downregulation in tubular cells

Given the good correlation between the expression of PGC-1α and its target genes *in vivo*, we explored whether TWEAK-induced PGC-1α downregulation results in reduced expression of genes encoding mitochondrial proteins in cultured tubular cells. In tubular cells, TWEAK decreased the gene expression of Ndufs1, Sdha, and Tfam (Figure 5a). Enforced PGC-1α overexpression achieved by adenoviral infection

Table 1 | Relative mRNA expression of PGC-1α targets in murine AKI versus control kidneys and in murine proximal tubular epithelial cells stimulated with TWEAK versus control

Gene symbol	Gene description	Mouse kidney (AKI/control ratio)	*P*-value	Murine proximal tubular cells (TWEAK/control ratio)	*P*-value
Ppargc1β	Peroxisome proliferative-activated receptor-γ coactivator-1β	0.595 ± 0.29	0.001	1.067 ± 0.014	NS
Ndufs1	**NADH dehydrogenase (ubiquinone) Fe-S protein 1**	**0.644 ± 0.3**	**0.0001**	**0.608 ± 0.13**	**0.005**
Ndufb5	NADH dehydrogenase (ubiquinone) 1β subcomplex, 5	0.676 ± 0.12	0.0002	1.053 ± 0.09	NS
Sdha	**Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)**	**0.751 ± 0.43**	**0.002**	**0.689 ± 0.11**	**0.012**
Esrra	Estrogen-related receptor-α	0.764 ± 0.79	0.012	1.06 ± 0.05	NS
Ppara	Peroxisome proliferator-activated receptor-α	0.769 ± 0.89	NS	0.972 ±0.03	NS
Cox5b	Cytochrome *c* oxidase, subunit Vb	0.807 ± 0.61	0.014	0.985 ± 0.03	NS
Tfam	**Transcription factor A, mitochondrial**	**0.808 ± 0.78**	**0.04**	**0.673 ± 0.04**	**0.005**
Atp5o	ATP synthase, H+ transporting, mitochondrial F1	0.859 ± 0.7	0.048	0.997 ± 0.03	NS

AKI, acute kidney injury; NS, not significant; PGC-1α, peroxisome proliferator-activated receptor-γ coactivador-1α. Bold values indicate *P*-value AKI versus control or TWEAK versus control.

(Figure 5b) prevented TWEAK-induced down-regulation of Ndufs1, Sdha, and Tfam (Figure 5c), suggesting that, indeed, TWEAK downregulation of PGC-1α led to downregulation of PGC-1α-dependent genes encoding mitochondrial proteins. Moreover, TWEAK caused loss of mitochondrial membrane potential (MMP) as assessed by decreased tetramethylrhodamine methyl ester staining (Figure 6a and b). As loss of MMP is also a feature of apoptotic cell death, we measured hypodiploid cells and observed that TWEAK did not induce cell death (Figure 6c). This is in line with prior detailed characterization of TWEAK actions in nonstressed tubular cells describing increased cell proliferation and no effect on cell viability.[23] PGC-1α overexpression as a result of adenovirus (Ad-PGC-1α) infection prevented TWEAK-induced MMP

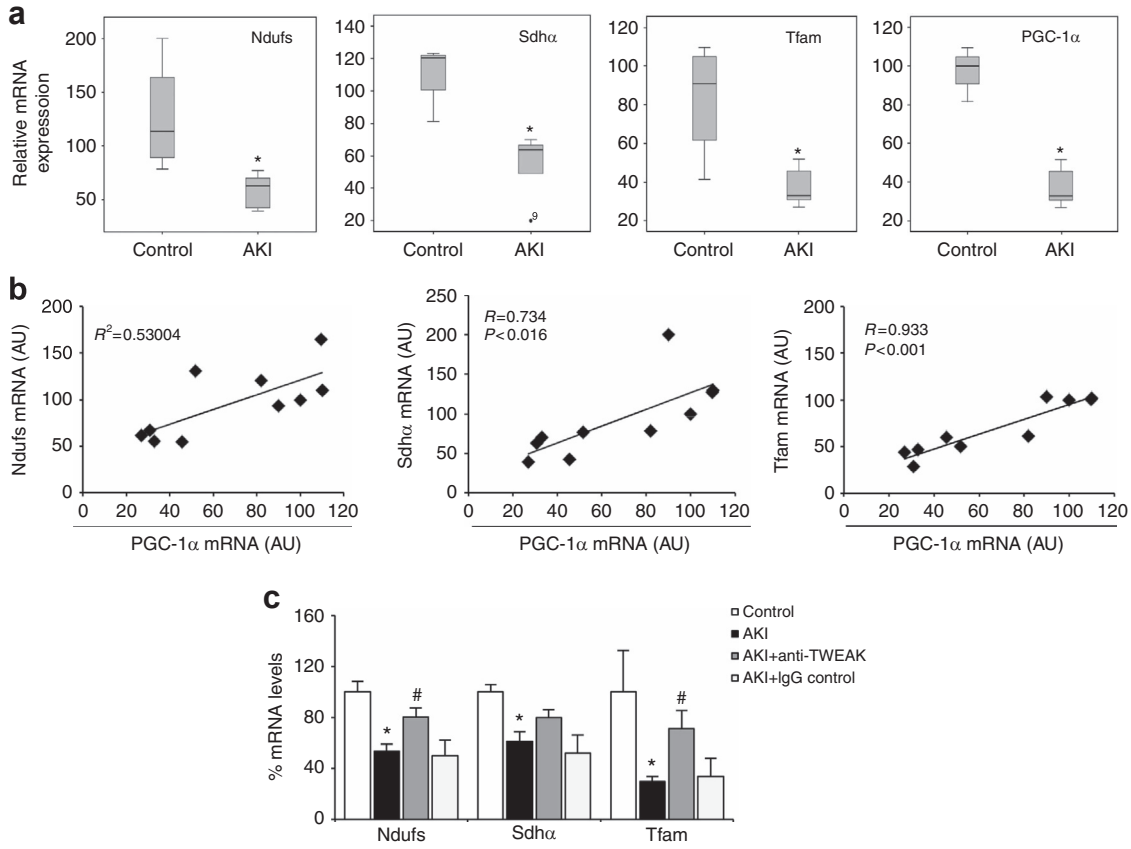

Figure 2 | TWEAK neutralization preserves the expression of PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) target genes during acute kidney injury (AKI). (a) Quantitative reverse transcriptase–PCR (RT-PCR) analyses of kidney PGC-1α and PGC-1α-regulated genes *Ndufs1, Sdha,* and *Tfam* in mice with AKI at 72 h. Mean ± SEM of five animals per group. *$P < 0.05$ versus control. **(b)** Scatter plot showing the significant positive correlation between kidney mRNA expression for PGC-1α and Ndufs1, Sdha, or Tfam in mice with AKI at 72 h ($n = 10$). **(c)** TWEAK neutralization prevented Ndufs1, Sdha, and Tfam mRNA downregulation in kidneys at 72 h after injury. Mean ± SEM of 5–6 animals per group. *$P < 0.02$ versus control, #$P < 0.03$ versus AKI with/without immunoglobulin G (IgG) control.

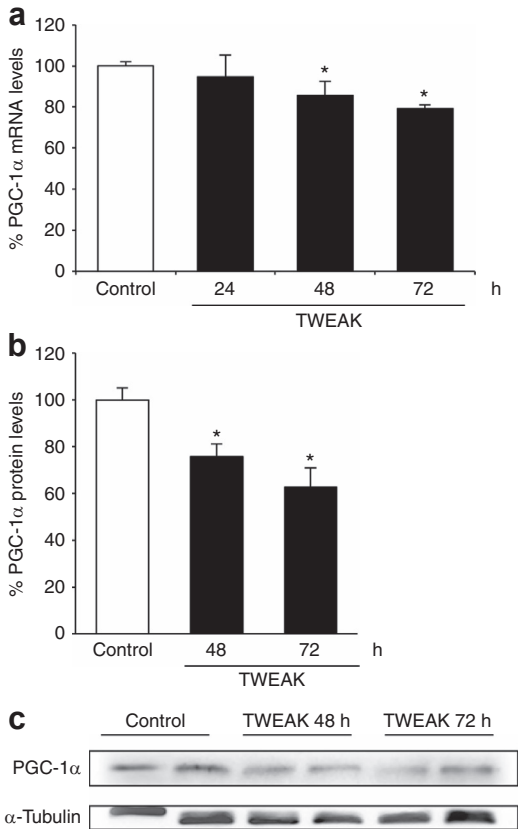

Figure 3 | TWEAK decreases PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) in kidneys from healthy mice. (a) Quantitative reverse transcriptase–PCR (RT-PCR) analyses of renal PGC-1α mRNA in mice 24, 48, or 72 h after systemic TWEAK administration. Mean ± SEM of five animals per group. *$P < 0.05$ versus control. **(b)** Total kidney PGC-1α protein levels in mice treated with TWEAK for 48 or 72 h measured by western blot. Mean ± SEM of five animals per group. *$P < 0.05$ versus control. **(c)** Representative western blot.

loss (Figure 6d). These results indicate that TWEAK may induce mitochondrial injury by downmodulating PGC-1α levels.

NF-κB mediates TWEAK-induced PGC-1α downregulation

Next, we explored the mechanisms that mediate TWEAK-induced PGC-1α downregulation. In tubular cells TWEAK activates the canonical NF-κB pathway that mediates multiple effects of TWEAK, such as increased expression of inflammatory cytokines and decreased expression of the anti-aging protein Klotho.[9,20] We now confirmed TWEAK activation of the canonical NF-κB pathway by demonstrating nuclear translocation or RelA (Supplementary Figure S4 online). In this regard, the canonical NF-κB inhibitor parthenolide[24] completely prevented nuclear translocation or RelA and the expression of NF-κB-dependent genes such as MCP-1 (Supplementary Figures S4 and S5 online)[9,16] and PGC-1α downregulation induced by TWEAK at the mRNA and protein levels (Figure 7a and b), suggesting that reduction in PGC-1α expression by TWEAK is mediated by NF-κB activation. Moreover, TWEAK induced RelA binding to the PGC-1α promoter as assessed by chromatin immunoprecipitation (ChIP; Figure 7c).

TWEAK regulates PGC-1α expression through epigenetic mechanisms

Histone acetylation/deacetylation has a key role in the regulation of gene expression. Indeed, histone deacetylation mediated by histone deacetylase (HDAC) activity is required for p65/RelA-dependent repression of Klotho gene expression induced by TWEAK in kidney tubular cells.[20] In this regard, pretreatment of tubular cells with HDAC inhibitors, trichostatin A or valproic acid, prevented PGC-1α downregulation induced by TWEAK at the mRNA and protein levels (Figure 8a and b). ChIP assays using antibodies against acetylated histones confirmed that TWEAK promotes histone H3 deacetylation at NF-κB-binding sites at the murine PGC-1α promoter in renal tubular cells, and this is prevented with trichostatin A pretreatment (Figure 8c).

DISCUSSION

The main findings of this study are that the expression levels of PGC-1α and PGC-1α target genes encoding mitochondrial proteins are downregulated in AKI *in vivo,* and this is dependent on the cytokine TWEAK. TWEAK also reduces the expression of PGC-1α and target genes in cultured tubular cells through NF-κB activation and histone deacetylation, resulting in impaired mitochondrial function.

PGC-1α is a transcriptional coactivator expressed in mitochondria-enriched tissues with high energy requirement such as liver, adipose tissue, pancreas, muscle, kidney, and the brain.[25] PGC-1α regulates mitochondrial biogenesis and associates with nuclear respiratory factors and mitochondrial transcription factor A (Tfam) to promote transcription of nuclear genes encoding mitochondrial proteins and mitochondrial biogenesis.[13,26,27] Studies of PGC-1α overexpression or loss of function support a role for PGC-1α in diseases such as heart failure and skeletal muscle atrophy.[28–30] There is evidence for a protective role of PGC-1α in kidney disease: (1) kidneys and proximal tubular cells are rich in mitochondria, and mitochondrial changes are a feature of AKI; (2) kidney PGC-1α levels are reduced in different models of AKI; and (3) PGC-1α overexpression or activation improves renal function.[4,6–8,31] Indeed, we observed that PGC-1α downregulation during AKI correlated with severity of renal failure and was associated with reduced expression of PGC-1α target genes encoding mitochondrial proteins. Interestingly, expression of the TWEAK receptor Fn14 negatively correlated with PGC-1α expression, suggesting a relation between PGC-1α reduced levels and TWEAK/Fn14 activation in AKI. Upregulation of Fn14 expression is the main pathway leading to increased activity of the TWEAK/Fn14 system, and TWEAK/Fn14 targeting protects from diverse features of AKI (inflammation, cell death, and renal dysfunction) in several experimental models of AKI.[9,10,14,16,23]

TWEAK mediates PGC-1α downregulation during AKI, and TWEAK reduced kidney cell PGC-1α *in vitro* and *in vivo.*

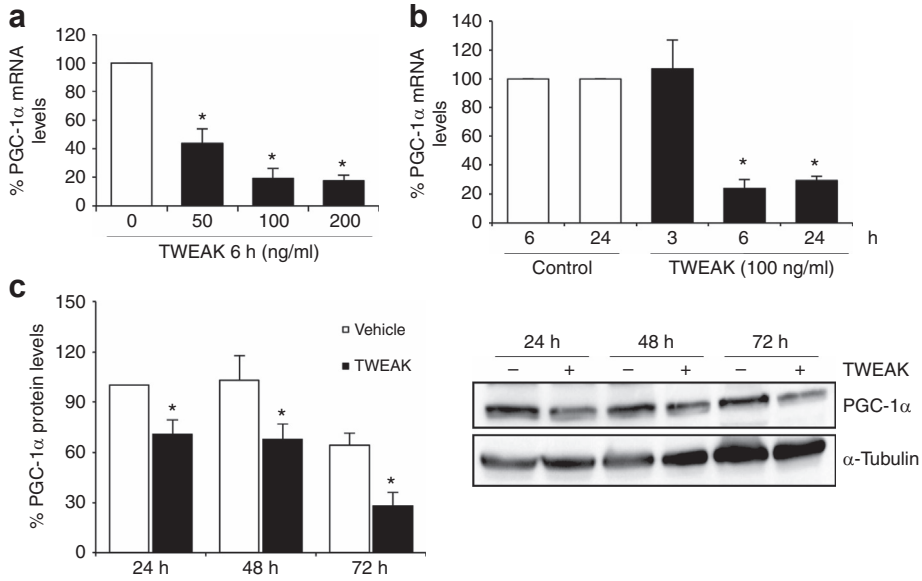

Figure 4 | TWEAK decreases PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) levels in cultured proximal tubular cells. (a) TWEAK decreases PGC-1α mRNA levels (reverse transcriptase–PCR [RT-PCR]) in a dose-dependent manner at 6 h in tubular cells. Mean ± SEM of three independent experiments. *$P < 0.05$ versus control. **(b)** Time course of PGC-1α mRNA levels (RT-PCR) in response to 100 ng/ml TWEAK in tubular cells. Mean ± SEM of three independent experiments. *$P < 0.05$ versus control. **(c)** Time course of total PGC-1α protein levels in tubular cells incubated with 100 ng/ml TWEAK for different time periods. Western blot. Mean ± SEM of four independent experiments. *$P < 0.05$ versus control.

These data are consistent with previous reports that TWEAK decreased PGC-1α levels in heart failure and skeletal muscle atrophy disease.[17,18]

The time course of TWEAK-induced PGC-1α downregulation is somewhat faster in cultured cells than the decrease in kidney PGC-1α following TWEAK administration *in vivo*. There are several potential explanations for this discrepancy, including a different latency time from administration of TWEAK to cell exposure to the cytokine, and the more complex *in vivo* environment in terms of multiple cell types, tissue architecture, and others. However, the delayed kidney PGC-1α response to TWEAK stimulation in healthy mice is consistent with both AKI data and limited experience in other cells and tissues. Thus, in AKI, both early (24 h) PGC-1α downregulation and renal dysfunction are TWEAK independent and probably a consequence of the

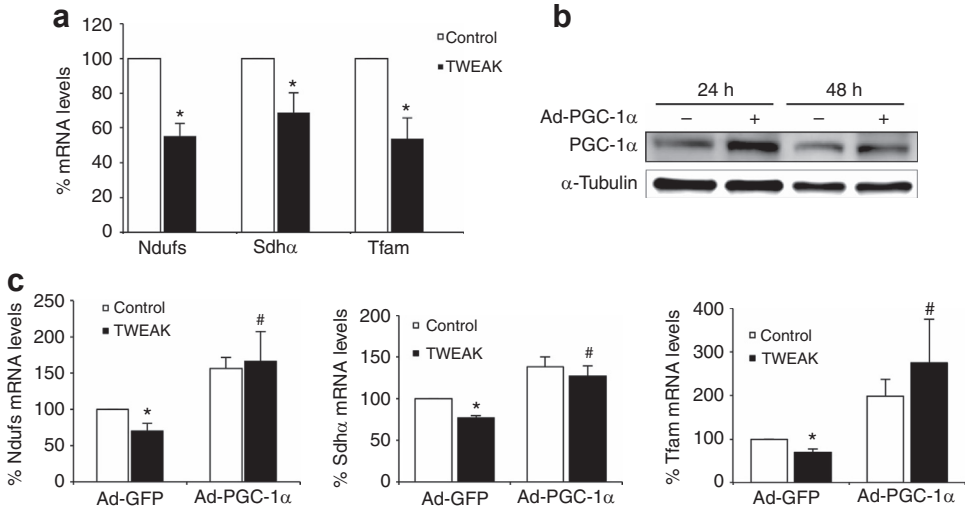

Figure 5 | TWEAK-induced downregulation of PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) results in reduced expression of genes encoding mitochondrial proteins. (a) Expression levels for mitochondrial genes *Ndufs1, Sdha,* and *Tfam* in tubular cells stimulated with 100 ng/ml TWEAK for 48 h (reverse transcriptase–PCR [RT-PCR]). Mean ± SEM of three independent experiments. *$P < 0.05$ versus control media. **(b)** PGC-1α expression is upregulated in tubular cells infected with Ad-PGC-1α compared with Ad-GFP cells. Western blot representative of three independent experiments. **(c)** Ad-PGC-1α restores the levels of mitochondrial genes reduced by TWEAK. Mean ± SEM of three independent experiments. *$P < 0.03$ versus control media; #$P < 0.05$ versus TWEAK+Ad-GFP.

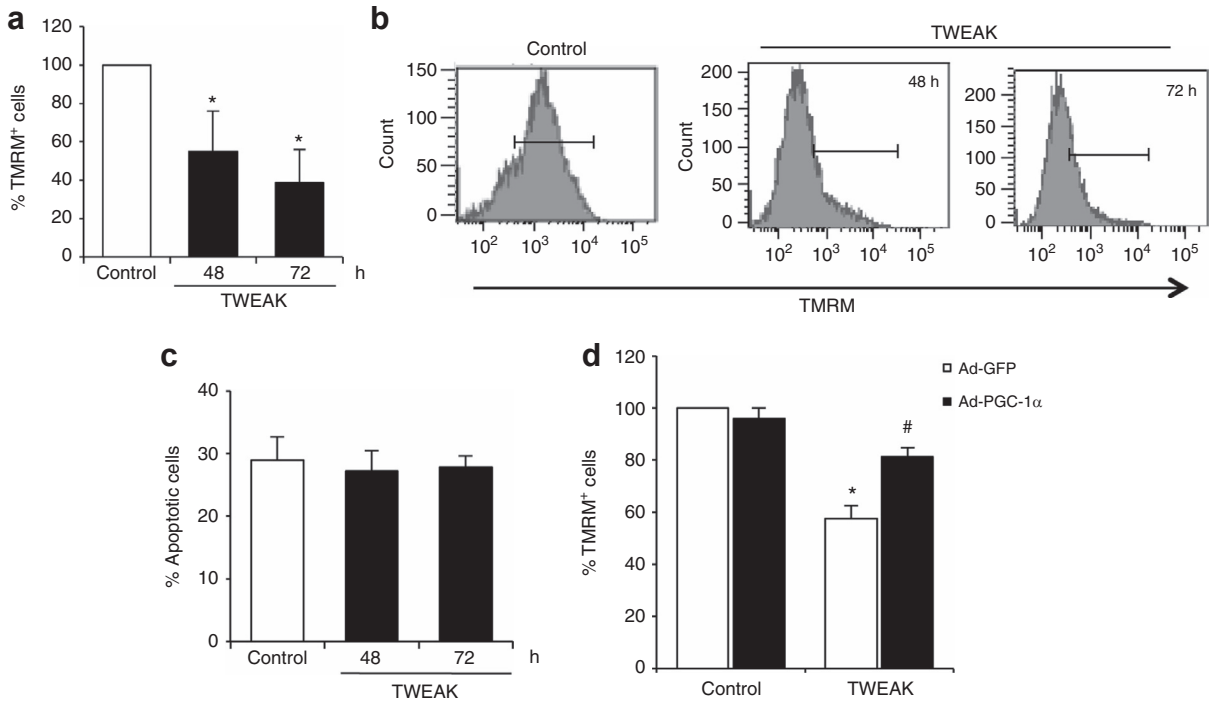

Figure 6 | TWEAK decreases the mitochondrial membrane potential (MMP) in tubular cells. (**a**) Tubular cells were incubated with 100 ng/ml TWEAK for 48 and 72 h. Cells were stained with tetramethylrhodamine methyl ester (TMRM), and the MMP was analyzed by flow cytometry. Mean ± SEM of four independent experiments. *$P < 0.05$ versus control. (**b**) Representative histogram of TMRM staining in tubular cells treated with TWEAK. Note decreased TMRM staining in cells stimulated with TWEAK. (**c**) Incubation for 48 and 72 h with 100 ng/ml TWEAK did not promote apoptotic cell death. Cells were permeabilized and stained with propidium iodide to assess hypodiploid cells by flow cytometry. Mean ± SEM of four independent experiments. (**d**) PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) overexpression prevents the decrease in MMP (TMRM staining) in tubular cells treated with TWEAK. Mean ± SEM of three independent experiments. *$P < 0.003$ versus control, #$P < 0.05$ versus TWEAK-Ad-GFP.

initial insult, despite TWEAK and Fn14 upregulation. In contrast, at later time points (72 h), both kidney failure and PGC-1α downregulation are TWEAK dependent. A similar discrepancy in the time course between cell culture and *in vivo* conditions has been observed in skeletal muscle. TWEAK decreased PGC-1α mRNA within 24 h in cultured myotubes.[18] However, following *in vivo* denervation, despite the early rise in Fn14 expression, the initial decrease in PGC-1α mRNA is TWEAK independent, but the subsequent, later (in this case 10 days) decrease in PGC-1α levels is TWEAK dependent.[18] In this regard, TWEAK induces persistent NF-κB activation.[32]

The physiological relevance of TWEAK-induced PGC-1α downregulation is supported by the fact that TWEAK also decreased the expression genes encoding mitochondrial proteins and led to loss of MMP. This is consistent with the hypothesis that PGC-1α downregulation contributes to TWEAK-mediated kidney dysfunction. Furthermore, enforced overexpression of PGC-1α prevented TWEAK-induced downregulation of PGC-1α-dependent genes. Among the genes encoding mitochondrial proteins down-regulated in AKI *in vivo* and by TWEAK in cultured cells we find Ndufs1, Sdha, and Tfam, encoding NADH dehydrogenase (ubiquinone) Fe-S protein 1 (NDUFS1), succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (SDHA), and mitochondrial transcription factor A (TFAM), respectively. NDUFS1 is a component of complex I of the respiratory chain, and Ndufs1 mutations cause neurological disease.[33,34] SDHA is the main catalytic subunit of mitochondrial complex II and is also a component of a mitochondrial adenosine triphosphate–sensitive potassium channel.[35] TFAM is a key mitochondrial transcription factor that regulates mitochondrial DNA replication and repair.[36]

TWEAK is a member of the TNF superfamily. Neutralizing anti-TNF antibodies are approved for clinical use in a variety of inflammatory conditions.[37] Although TNF actions on kidney cells have been extensively characterized, and they continue to be studied, much less is known about TWEAK actions on kidney cells. Indeed, some basic differences are already known. Thus, TNF promotes death in non-stressed kidney tubular cells,[38] whereas TWEAK promotes cell proliferation.[23] TNF promotes transient canonical NF-κB pathway activation and chemokine expression, whereas TWEAK promotes both transient canonical and persistent noncanonical NF-κB pathway activation with secretion of an only partially overlapping set of chemokines.[16] Neutralizing anti-TWEAK antibodies are undergoing clinical trials in lupus nephritis.[13] Thus, given the potential future availability of TWEAK targeting strategies, is it worth dissecting the different pathways activated by TWEAK in the setting of kidney injury. TWEAK

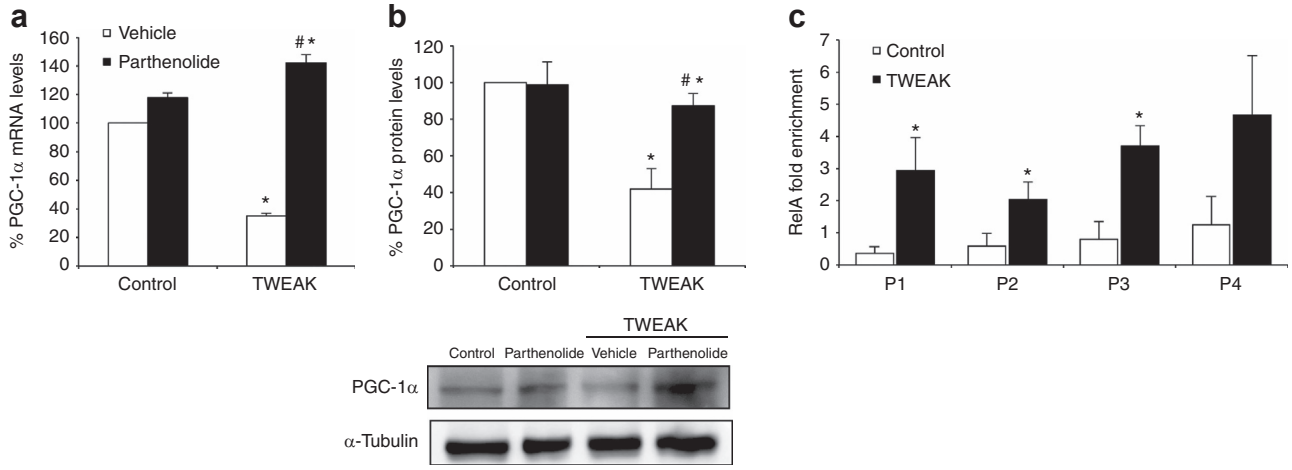

Figure 7 | Nuclear factor (NF)-κB mediates TWEAK-induced PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) downregulation. (a, b) The NF-κB inhibitor parthenolide (10 μmol/l) prevents TWEAK-induced PGC-1α downregulation in cultured tubular cells at 6 h at the **(a)** mRNA (reverse transcriptase–PCR [RT-PCR]) and **(b)** protein (western blot) levels. Mean ± SEM of three independent experiments. $^{*}P < 0.05$ versus control; $^{\#}P < 0.05$ versus TWEAK alone. **(c)** Tubular cells were stimulated with TWEAK for 6 h, and chromatin immunoprecipitation (ChIP) analyses were performed using an anti-NF-κB p65 (RelA) antibody. Promoter copy number was quantified by real-time PCR in duplicate using four specific primers that amplify four different NF-κB-binding sites of the PGC-1α promoter. Normal rabbit immunoglobulin G (IgG) was used as negative control for the specificity of the immunoprecipitation (IP). As a positive control, aliquots of chromatin fragments obtained before IP were also subjected to RT-PCR analysis (input). DNA immunoprecipitated with RelA was normalized to a 100-fold dilution of input chromatin. Data are expressed as fold enrichment of RelA binding compared with negative control antibody (normal rabbit IgG). Mean ± SEM of three independent experiments.

decreased MMP without inducing cell death. However, TWEAK is known to sensitize to cell death in conjunction with other stimuli, such as proinflammatory milieu[39] or uremic toxins.[40] The decreased PGC-1α and mitochondrial dysfunction induced by TWEAK may contribute to sensitization to the lethality of other stimuli.

TWEAK and NF-κB pathway are well-known activators of the inflammatory response. In addition, the present results suggest that they may further promote kidney injury in cooperation with histone deacetylation by decreasing the expression of factors with a cell protective function, including PGC-1α and, as previously described, Klotho,[20] that prevents stress-induced senescence and has anti-inflammatory and antifibrotic functions.[19] Histone deacetylation had a key role in downregulating PGC-1α expression. This effect appeared to be dependent on histone deacetylation at NF-κB-binding sites at the PPARGC1A locus promoter as RelA bound to the same sites. We relied on inhibitors to interfere with TWEAK actions, and inhibitors may have nonspecific effects. However, two different HDAC inhibitors yielded similar results, and promotion of histone acetylation by trichostatin A was confirmed. Furthermore, parthenolide inhibition of NF-κB has been characterized in detail in this cell system.[9,21] In this regard, the clinical translation of these observations will likely come in the form of inhibitors. Thus, HDAC inhibitors are undergoing clinical trials and potentially have multiple gene targets. An improved understanding of the range of targets may help further refine this therapeutic approach.

Figure 9 summarizes our current hypothetical mechanism for TWEAK-induced PGC-1α downregulation. On the basis of the current results and the evolving understanding of the NF-κB system, we hypothesize that NF-κB RelA binding to the PGC-1α promoter may recruit HDAC corepressor proteins that promote histone deacetylation and chromatin condensation, thus repressing gene expression. The transactivation function of NF-κB has long been known to be regulated in part through the association of NF-κB RelA with HDAC corepressor proteins.[41] This was initially described as a mechanism that limited the upregulation of the expression of NF-κB-regulated genes and controlled the induced level of expression of these genes.[41] However, in recent years, several examples have been described of NF-κB-mediated gene suppression through NF-κB RelA recruitment of HDAC and subsequent decreased histone acetylation and repressed gene expression.[42–44] These include oxygen radical–induced downregulation of TRPC6 expression in mesangial cells,[42] platelet-derived growth factor-D repression by interleukin-1β in smooth muscle cells,[43] and TNF-α-induced suppression of YKL-40 expression in glioma cells.[44]

In summary, the current work suggests that TWEAK/Fn14 signaling represses PGC-1α expression during AKI through activation of canonical NF-κB pathways and epigenetic mechanisms including histone deacetylation on NF-κB-binding sites. These results may be relevant to design therapeutic approaches to upregulate PGC-1α expression during kidney injury.

MATERIALS AND METHODS

Animal models

All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and

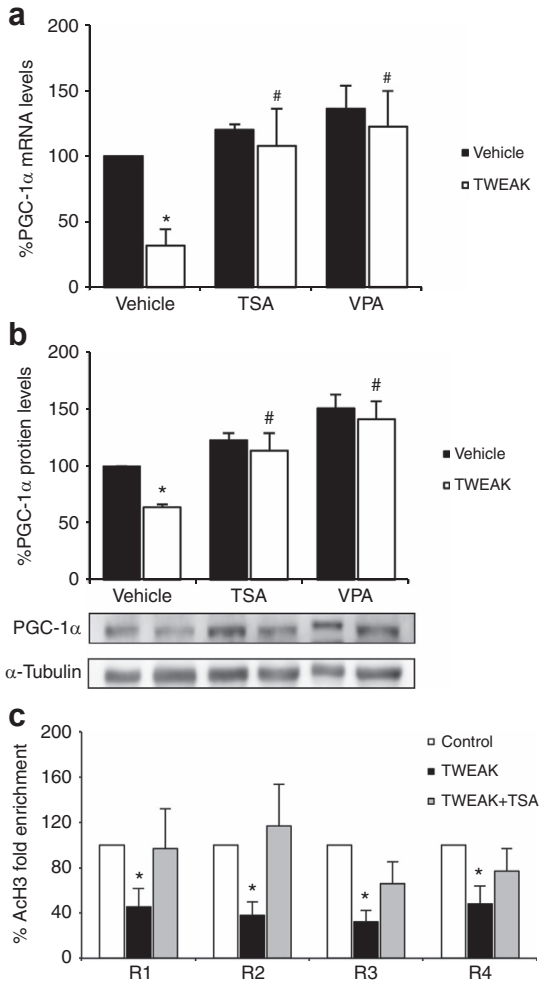

Figure 8 | TWEAK-induced PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) downregulation required histone deacetylase (HDAC) activity. The HDAC inhibitors trichostatin A (TSA) and valproic acid (VPA) prevent downregulation of PGC-1α expression in tubular cells stimulated with TWEAK at 6 h. Cells were prestimulated with TSA (1.5 ng/ml) or VPA (500 μmol/l) 1 h before addition of TWEAK. **(a)** PGC-1α mRNA was assessed by reverse transcriptase–PCR (RT-PCR). Mean ± SEM of four independent experiments. $^{*}P < 0.05$ versus control, $^{\#}P < 0.05$ versus TWEAK alone. **(b)** Representative western blot and quantification of three independent experiments of PGC-1α expression in cells treated with HDAC inhibitors and TWEAK. $^{*}P < 0.04$ versus control; $^{\#}P < 0.05$ versus TWEAK alone. **(c)** Tubular cells were pretreated with TSA (1.5 ng/ml) for 1 h and stimulated with TWEAK for 6 h, and chromatin immunoprecipitation (ChIP) analyses were performed using an anti-acetylated histone H3 antibody (AcH3). Promoter copy number was quantified by real-time PCR in duplicate using four specific primers that amplify four different nuclear factor (NF)-κB-binding sites of the PGC-1α promoter. Normal rabbit immunoglobulin G (IgG) was used as negative control for the specificity of the immunoprecipitation (IP). As a positive control, aliquots of chromatin fragments obtained before IP were also subjected to RT-PCR analysis (Input). DNA immunoprecipitated with AcH3 was normalized to a 100-fold dilution of input chromatin. Data are expressed as fold enrichment of AcH3 binding compared with negative control antibody (normal rabbit IgG). Mean ± SEM of three independent experiments.

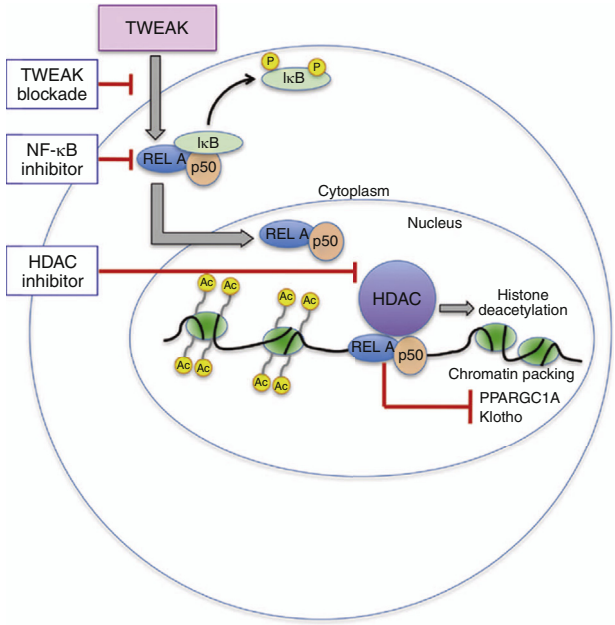

Figure 9 | Hypothetical model for TWEAK-induced, nuclear factor (NF)-κB-mediated downregulation of PGC-1α (peroxisome proliferator-activated receptor-γ coactivador-1α) expression: recruitment of histone deacetylase (HDAC). Data presented here are consistent with the hypothesis that upon TWEAK stimulation, NF-κB RelA directly binds to the promoter of the *PPARGC1A* gene encoding PGC-1α to recruit, as described for other cell systems,[41] HDAC corepressor proteins, thus leading to histone deacetylation and chromatin packing that contributes to suppressed gene expression. This mechanism may also lead to Klotho downregulation.[20]

were approved by the animal ethics committee of IIS-FJD (Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz). C57/BL/6 female mice (12 weeks old; Charles River, Chatillon-sur-Charlaronne, France) were treated intraperitoneally with 25 μg/kg TWEAK (Alexis, Enzo Life Sciences, Laüfelfingen, Switzerland) or its vehicle (saline), and mice were killed 24, 48, or 72 h later ($n = 5$ per group). The dose of TWEAK was calculated based on *in vitro* experiments for an extracellular volume of 6.5 ml per mouse and was previously shown to induce biological responses in kidneys.[9]

Folic acid nephropathy is a classical model of kidney tubulointerstitial injury and inflammation.[9] C57BL/6 mice (12–14 weeks old) received a single intraperitoneal injection of folic acid (Sigma, St Louis, MO) 300 mg/kg in 0.3 mol/l sodium bicarbonate or vehicle, and mice were killed 6, 24, and 72 h, or 7 days later ($n = 5$). In a second experiment, mice were dosed intraperitoneally with 200 μg neutralizing anti-TWEAK monoclonal antibody (clone P2D10, Biogen Idec, Cambridge, MA) or isotype control immunoglobulin G (IgG)[20,21] ($n = 5$–6 per group). Animals received the monoclonal antibody 1 day before and 2 days after folic acid injection, and they were killed 24 and 72 h after folic acid injection. Kidneys were perfused *in situ* with cold saline before removal. One kidney was snap frozen in liquid nitrogen for RNA and protein studies and the other fixed and paraffin embedded.

Transcriptomics arrays

Transcriptomics arrays of kidney tissue ($n = 3$ kidneys obtained 24 h after folic acid and $n = 3$ obtained 24 h after vehicle acid administration to mice) and MCT tubular cells exposed to 100 ng/ml TWEAK for 6 h or vehicle ($n = 3$ independent experiments) were performed at Unidad Genómica Moncloa, Fundación Parque Científico de Madrid, Madrid, Spain. Affymetrix (Santa Clara, CA)

microarray analysis followed the manufacturer's protocol.[20,21] Image files were initially obtained through Affymetrix GeneChip Command Console Software (AGCC). Subsequently, Robust Multichip Analysis was performed using Affymetrix Expression Console Software. Starting from the normalized Robust Multichip Analysis, the Significance Analysis of Microarrays was performed using the limma package (Babelomics, www.babelomics.org), using a false discovery rate of 5% to identify genes that were significantly differentially regulated between the analyzed groups.

Cells and reagents

MCT murine proximal tubular epithelial cells are a cell line originally harvested from the cortex of SJL mice and extensively characterized.[45,46] Cells were cultured in RPMI-1640 with 10% fetal bovine serum, 2 mmol/l glutamine, and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in 5% CO_2 at 37°C. RPMI-1640, penicillin, streptomycin, and trypsin-EDTA were from Bio-Whittaker (Waltham, MA) and fetal bovine serum from Gibco (Carlsbad, CA). For experiments, cells were serum depleted for 24 h and then stimulated with different molecules. Recombinant human TWEAK (Millipore, Billerica, MA), unless otherwise specified, was used at 100 ng/ml, based on prior dose–response studies performed in our lab.[9,23] The HDAC inhibitors trichostatin A (Upstate Biotechnology, Millipore) and valproic acid (Sigma-Aldrich, St Louis, MO) were used at 1.5 ng/ml and 500 μmol/l, respectively. The NF-κB inhibitor parthenolide (Sigma) was used at 10 μmol/l based on previous dose–response studies in this system.[9] For adenovirus experiments, MCT cells were infected with a green fluorescent protein control virus or an adenovirus encoding murine PGC-1 at 48 h before the experiment. Efficiency was confirmed by green fluorescent protein visualization and PGC-1α expression by western blot.

RNA extraction and real-time PCR

Total RNA was extracted from cells by the TRI Reagent method (Invitrogen, Paisley, UK), and 1 μg RNA was reverse transcribed with the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). All assays were from Applied Biosystems. Quantitative PCR was performed by 7500 Real-Time PCR System with the Prism 7000 System SDS Software (Applied Biosystems), and RNA expression of different genes was corrected for glyceraldehyde 3-phosphate dehydrogenase (GAPDH).[9]

Western blot

Cell samples were homogenized in lysis buffer (50 mmol/l Tris HCl, 150 mmol/l NaCl, 2 mmol/l EDTA, 2 mmol/l EGTA, 0.2% Triton X-100, 0.3% NP-40, 0.1 mmol/l phenylmethylsulfonyl fluoride, and 1 μg/ml pepstatin A) and then separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions. After electrophoresis, samples were transferred to polyvinylidene difluoride membranes (Millipore), blocked with 5% skimmed milk in phosphate-buffered saline (PBS)/0.5% v/v Tween-20 for 1 h, washed with PBS/Tween, and incubated with mouse polyclonal anti-PGC-1α (1:1000, CalBiochem, La Jolla, CA) diluted in 5% milk PBS/Tween, anti-Fn14 (1:1000, Cell Signalling, Danvers, MA), or anti-TWEAK (1:1000, Novus Laboratories, Bristol, VA) diluted in 5% bovine serum albumin (BSA) in PBS/Tween. Blots were washed with PBS/Tween and incubated with appropriate horseradish peroxidase–conjugated secondary antibody (1:2000, Amersham, Aylesbury, UK). After washing with PBS/Tween, blots were developed with the chemiluminescence method (ECL) (Amersham) and probed with mouse monoclonal anti-α-tubulin antibody (1:2000, Sigma). Levels of expression were corrected for minor differences in loading.

Immunofluorescence

Kidney tissue immunofluorescence was performed in 3 μm thick sections of paraffin-embedded tissue using a PT-link device (with a low pH solution for TWEAK and a high pH solution for Fn14, 95°C, 20 min). Sections were then washed with PBS for 5 min and blocked by incubation with 4% PBS/BSA+6% serum. For immunostaining, sections were incubated with anti-Fn14 (1:50 in 4% PBS/BSA+1% serum, Cell Signalling) or anti-TWEAK (1:10 in 4% PBS/BSA+2% serum, R&D, Minneapolis, MN) at 4 °C overnight, followed by incubation with Alexa Fluor 633-goat anti-rabbit IgG for Fn14 and Alexa Fluor 633-rabbit anti-goat for TWEAK (1/200 in 4% BSA/PBS, Invitrogen) for 1 h at 37 °C. Then, tissues were incubated with fluorescein-conjugated Lotus tetragonolobus lectin (Vector Labs, Burlingame, CA) for proximal tubules staining or Dolichos biflorus lectin (Sigma) for distal tubules staining (1/30 in 4% BSA/PBS) for 30 min at room temperature, counterstained with 4′,6-diamidino-2-phenylindole, and mounted. Negative controls included incubation with isotype IgG.

Mitochondrial membrane potential

Changes in MMP were determined as differences in tetramethylrhodamine methyl ester fluorescence (Molecular Probes, Life Technologies, Foster City, CA). Adherent cells were pooled with spontaneously detached cells and stained with 150 nmol/l tetramethylrhodamine methyl ester for 10 min at 37 °C. Fluorescence intensity was measured by flow cytometry using BD FACS Diva Software (BD Biosciences, San Jose, CA). Decreased tetramethylrhodamine methyl ester fluorescence indicates that MMP is reduced.

Characterization of apoptotic cells

For assessment of apoptosis by flow cytometry, adherent cells were pooled with spontaneously detached cells and incubated in 100 μg/ml propidium iodide, 0.05% NP-40, and 10 μg/ml RNAse A in PBS at 4 °C for >1 h. This assay permeabilizes the cells, and thus propidium iodide stains both live and dead cells. The percentage of apoptotic cells with decreased DNA staining (hypodiploid cells) was counted flow cytometry using BD FACS Diva Software (BD Biosciences).

ChIP assay

To perform ChIP assays, we used 0.5 to 1 × 10^6 cells per sample as described previously.[20] In brief, cells fixed with 35% formaldehyde were lysed in Lysis Buffer (5 mmol/l HEPES, 85 mmol/l KCl, 0.5% NP-40, pH 8.0, plus protease inhibitor cocktail), and the pellet was resuspended in Nuclei Lysis Buffer (50 mmol/l Tris-HCl, 10 mmol/l EDTA, and 1% sodium dodecyl sulfate, pH 8.1) and sonicated in a Diagenode sonicator (Denville, NJ). For ChIP, anti-rabbit IgG Dynabeads (Life Technologies, Foster City, CA) were used. Chromatin was diluted into ChIP Binding Buffer (10% Triton-X, 10% sodium deoxycholate, TE plus protease inhibitor cocktail) and immunoprecipitated using IgG Dynabeads with 3 μg anti-acetyl-Histone H3 (Lys9) (Merck Millipore, Billerica, MA) antibody or anti-NF-κB p65 (RelA) (Merck Millipore) antibody. Antibody-chromatin complexes were then washed and eluted from beads using Elution Buffer (1 mol/l Tris (pH 8.0), 0.5 mol/l EDTA, 10% sodium dodecyl sulfate, and dH_2O). After crosslink reversal and

proteinase K treatment, DNA was extracted with phenol/chloroform, and ethanol was precipitated. DNA immunoprecipitated from 1 μl eluted DNA was analyzed in duplicate by real-time PCR. Primers were designed to amplify NF-κB (regions 1 and 4) at the promoter of the PGC-1α gene, identified by ALGGEN-PROMO (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). Aliquots of chromatin obtained before immunoprecipitation were analyzed as input control. Primers for each region are listed in Supplementary Table S1 online. Results are presented as fold enrichment of precipitated DNA associated with a given histone modification binding, relative to a 1:100 dilution of input chromatin.

Statistics

Statistical analysis was performed using the SPSS 11.0 statistical software (Chicago, IL). Results are expressed as mean ± SEM. Significance at the $P < 0.05$ level was assessed by nonparametric Mann–Whitney *U*-test for two groups and analysis of variance (Fisher least significant difference *post hoc* test) for three or more groups. Pearson's correlation was used to assess correlation between two continuous variables.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Renal function parameters in folic acid–induced AKI.
Figure S2. Increased kidney TWEAK and Fn14 expression in folic acid–induced AKI.
Figure S3. Effect of TWEAK neutralization on early kidney dysfunction.
Figure S4. Parthenolide inhibits TWEAK-induced NFκB activation.
Figure S5. NFκB mediates TWEAK-induced PGC-1α downregulation.
Table S1. Primers for ChIP (chromatin immunoprecipitation) real-time RT-PCR.
Supplementary material is linked to the online version of the paper at www.kidney-international.org.

REFERENCES

1. Zhu C, Xuan X, Che R, et al. Dysfunction of the PGC-1α-mitochondria axis confers adriamycin-induced podocyte injury. *Am J Physiol Renal Physiol*. 2014;306:F1410–F1417.
2. Siew ED, Davenport A. The growth of acute kidney injury: a rising tide or just closer attention to detail? *Kidney Int*. 2015;87:46–61.
3. Bellomo R, Kellum JA, Ronco C. Acute kidney injury. *Lancet*. 2012;380:756–766.
4. Tábara LC, Poveda J, Martin-Cleary C, et al. Mitochondria-targeted therapies for acute kidney injury. *Expert Rev Mol Med*. 2014;16:e13.
5. Rato L, Duarte AI, Tomás GD, et al. Pre-diabetes alters testicular PGC1-α/SIRT3 axis modulating mitochondrial bioenergetics and oxidative stress. *Biochim Biophys Acta*. 2014;1837:335–344.
6. Stallons LJ, Whitaker RM, Schnellmann RG. Suppressed mitochondrial biogenesis in folic acid-induced acute kidney injury and early fibrosis. *Toxicol Lett*. 2014;224:326–332.
7. Funk JA, Schnellmann RG. Persistent disruption of mitochondrial homeostasis after acute kidney injury. *Am J Physiol Renal Physiol*. 2012;302:F853–F864.
8. Tran M, Tam D, Bardia A, et al. PGC-1α promotes recovery after acute kidney injury during systemic inflammation in mice. *J Clin Invest*. 2011;121:4003–4014.
9. Sanz AB, Justo P, Sanchez-Niño MD, et al. The cytokine TWEAK modulates renal tubulointerstitial inflammation. *J Am Soc Nephrol*. 2008;19:695–703.
10. Hotta K, Sho M, Yamato I, et al. Direct targeting of fibroblast growth factor-inducible 14 protein protects against renal ischemia reperfusion injury. *Kidney Int*. 2011;79:179–188.
11. Xia Y, Herlitz LC, Gindea S, et al. Deficiency of fibroblast growth factor-inducible 14 (fn14) preserves the filtration barrier and ameliorates lupus nephritis. *J Am Soc Nephrol*. 2015;26:1053–1070.
12. Sanz AB, Izquierdo MC, Sanchez-Niño MD, et al. TWEAK and the progression of renal disease: clinical translation. *Nephrol Dial Transplant*. 2014;29(Suppl 1):i54–i62.
13. Medvedeva NV, Prosorovskiy VN, Ignatov DV, et al. Pharmacological agents and transport nanosystems based on plant phospholipids. *Biomed Khim*. 2015;61:219–230.
14. Sanz AB, Sanchez-Niño MD, Ramos AM, et al. NF-kappaB in renal inflammation. *J Am Soc Nephrol*. 2010;21:1254–1262.
15. Poveda J, Tabara LC, Fernandez-Fernandez B, et al. TWEAK/Fn14 and non-canonical NF-kappaB signaling in kidney disease. *Front Immunol*. 2013;4:447.
16. Sanz AB, Sanchez-Niño MD, Izquierdo MC, et al. TWEAK activates the noncanonical NFkappaB pathway in murine renal tubular cells: modulation of CCL21. *PLoS One*. 2010;5:e8955.
17. Shi J, Jiang B, Qiu Y, et al. PGC1α plays a critical role in TWEAK-induced cardiac dysfunction. *PLoS One*. 2013;8:e54054.
18. Hindi SM, Mishra V, Bhatnagar S, et al. Regulatory circuitry of TWEAK-Fn14 system and PGC-1α in skeletal muscle atrophy program. *FASEB J*. 2014;28:1398–1411.
19. Zhou L, Li Y, Zhou D, et al. Loss of Klotho contributes to kidney injury by derepression of Wnt/β-catenin signaling. *J Am Soc Nephrol*. 2013;24:771–785.
20. Moreno JA, Izquierdo MC, Sanchez-Niño MD, et al. The inflammatory cytokines TWEAK and TNFa reduce renal klotho expression through NFκB. *J Am Soc Nephrol*. 2011;22:1315–1325.
21. Izquierdo MC, Sanz AB, Mezzano S, et al. TWEAK (tumor necrosis factor-like weak inducer of apoptosis) activates CXCL16 expression during renal tubulointerstitial inflammation. *Kidney Int*. 2012;81:1098–1107.
22. Jarr KU, Eschricht S, Burkly LC, et al. TNF-like weak inducer of apoptosis aggravates left ventricular dysfunction after myocardial infarction in mice. *Mediators Inflamm*. 2014;2014:131950.
23. Sanz AB, Sanchez-Niño MD, Izquierdo MC, et al. Tweak induces proliferation in renal tubular epithelium: a role in uninephrectomy induced renal hyperplasia. *J Cell Mol Med*. 2009;13:3329–3342.
24. Hehner SP, Hofmann TG, Dröge W, et al. The antiinflammatory sesquiterpene lactone parthenolide inhibits NF-kappa B by targeting the I kappa B kinase complex. *J Immunol*. 1999;163:5617–5623.
25. Besseiche A, Riveline JP, Gautier JF, et al. Metabolic roles of PGC-1α and its implications for type 2 diabetes. *Diabetes Metab*. 2015 (doi: 10.1016/j.diabet.2015.02.002; e-pub ahead of print).
26. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*. 1999;98:115–124.
27. Finck BN, Kelly DP. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest*. 2006;116:615–622.
28. Vainshtein A, Desjardins EM, Armani A, et al. PGC-1α modulates denervation-induced mitophagy in skeletal muscle. *Skelet Muscle*. 2015;5:9.
29. Arany Z, He H, Lin J, et al. Transcriptional coactivator PGC-1 alpha controls the energy state and contractile function of cardiac muscle. *Cell Metab*. 2005;1:259–271.
30. Russell LK, Mansfield CM, Lehman JJ, et al. Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1 alpha promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. *Circ Res*. 2004;94:525–533.
31. Funk JA, Schnellmann RG. Accelerated recovery of renal mitochondrial and tubule homeostasis with SIRT1/PGC-1α activation following ischemia-reperfusion injury. *Toxicol Appl Pharmacol*. 2013;273:345–354.

32. Sanz AB, Sanchez-Niño MD, Ortiz A. TWEAK, a multifunctional cytokine in kidney injury. *Kidney Int*. 2011;80:708–718.
33. Dieteren CE, Koopman WJ, Swarts HG, et al. Subunit-specific incorporation efficiency and kinetics in mitochondrial complex I homeostasis. *J Biol Chem*. 2012;287:41851–41860.
34. Petruzzella V, Sardanelli AM, Scacco S, et al. Dysfunction of mitochondrial respiratory chain complex I in neurological disorders: genetics and pathogenetic mechanisms. *Adv Exp Med Biol*. 2012;942: 371–384.
35. Hoekstra AS, Bayley JP. The role of complex II in disease. *Biochim Biophys Acta*. 2013;1827:543–551.
36. Kaufman BA, Durisic N, Mativetsky JM, et al. The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. *Mol Biol Cell*. 2007;18: 3225–3236.
37. Kalunian K, Merrill JT. New directions in the treatment of systemic lupus erythematosus. *Curr Med Res Opin*. 2009;25: 1501–1514.
38. Ortiz A, Lorz C, Catalán MP, et al. Expression of apoptosis regulatory proteins in tubular epithelium stressed in culture or following acute renal failure. *Kidney Int*. 2000;57:969–981.
39. Justo P, Sanz AB, Sanchez-Niño MD, et al. Cytokine cooperation in renal tubular cell injury: the role of TWEAK. *Kidney Int*. 2006;70: 1750–1758.
40. Poveda J, Sanchez-Niño MD, Glorieux G, et al. p-cresyl sulphate has pro-inflammatory and cytotoxic actions on human proximal tubular epithelial cells. *Nephrol Dial Transplant*. 2014;29:56–64.
41. Ashburner BP, Westerheide SD, Baldwin AS. The p65 (RelA) subunit of NF-kappaB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. *Mol Cell Biol*. 2001;21:7065–7077.
42. Wang Y, Ding M, Chaudhari S, et al. Nuclear factor κB mediates suppression of canonical transient receptor potential 6 expression by reactive oxygen species and protein kinase C in kidney cells. *J Biol Chem*. 2013;288:12852–12865.
43. Liu MY, Khachigian LM. Histone deacetylase-1 is enriched at the platelet-derived growth factor-D promoter in response to interleukin-1 beta and forms a cytokine-inducible gene-silencing complex with NF-kappab p65 and interferon regulatory factor-1. *J Biol Chem*. 2009;284:35101–35112.
44. Bhat KP, Pelloski CE, Zhang Y, et al. Selective repression of YKL-40 by NF-kappaB in glioma cell lines involves recruitment of histone deacetylase-1 and -2. *FEBS Lett*. 2008;582:3193–3200.
45. Haverty TP, Kelly CJ, Hines WH, et al. Characterization of a renal tubular epithelial cell line which secretes the autologous target antigen of autoimmune experimental interstitial nephritis. *J Cell Biol*. 1988;107: 1359–1368.
46. Strutz F, Okada H, Lo CW, et al. Identification and characterization of a fibroblast marker: FSP1. *J Cell Biol*. 1995;130:393–405.