THE DEFICIENCY OF THE TRANSCRIPTIONAL COACTIVATOR PGC-1α INCREASES RENAL INJURY IN ACUTE KIDNEY INJURY (AKI)

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INTRODUCTION & OBJECTIVES

Acute kidney injury (AKI) results in an acute and usually transient decrease in renal function, and it is associated with high mortality and a higher risk of developing chronic kidney disease. Currently, no satisfactory treatment attenuates AKI or accelerates recovery. AKI is characterized by an early event of tubular cell death followed by tubular dedifferentiation, proliferation and regeneration and this is accompanied by inflammation.

In recent years, a key role of **mitochondria** in AKI has been suggested. Moreover, during AKI there is evidence of mitochondrial injury and dysfunction. **PGC-1α** (Peroxisome proliferator-activated receptor gamma coactivador-1α), is a key transcriptional regulator of the expression of key mitochondrial proteins. Therefore, PGC-1α is involved in mitochondrial biogenesis, energy homeostasis, and oxidative stress. PGC-1α downregulation has been observed in experimental AKI and in renal cells treated with the cytokine TWEAK.

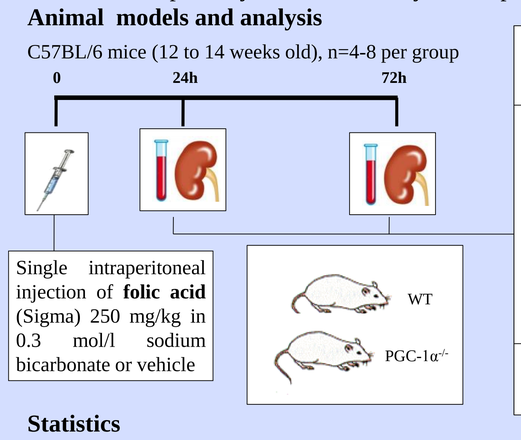
To further gain insights into the role of PGC1α in nephrotoxic AKI, we have explored for the first time the **consequences of PGC1α deficiency for nephrotoxicity**.

METHODS

Transcriptomics arrays and Pathway Enrichment

Transcriptomics arrays of control and AKI kidney mice were performed at Unidad Genómica Moncloa, Fundación Parque Científico de Madrid (Spain). Significance Analysis of Microarrays was performed using a false discovery rate (FDR) of 5%.Canonical pathway enrichment analysis was performed using Ingenuity Pathway Analysis (IPA).

Animal models and analysis

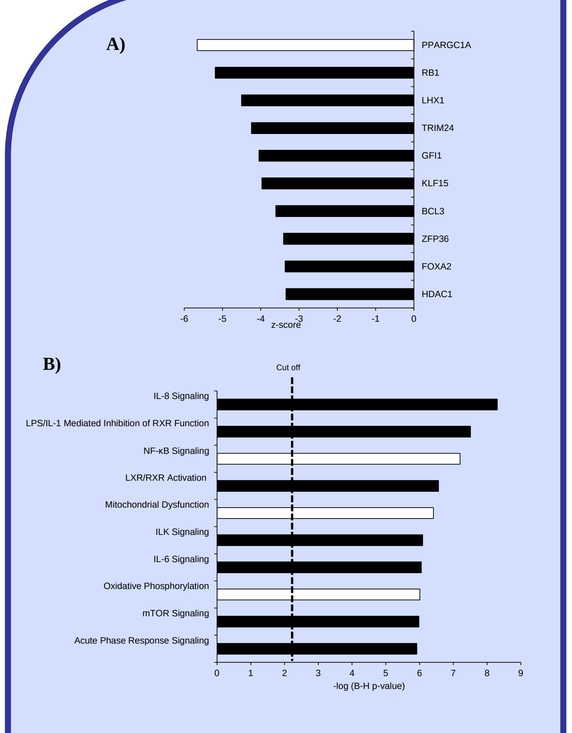


	RNA extraction, **retrotransciption** and **real-time polymerase chain reaction**
	Immunhistochemistry: anti-TOM22 (1:200, SIGMA), anti-PCNA (1:150, Santa Cruz), anti-CD3 (1:150, DAKO), and anti-F4/80 (1:70, BioRad). **TUNEL** (Terminal deoxynucleotidyl transferase dUTP nick end labeling) (Roche) **Southwestern**: sections incubated with 50 pmol of digoxigenin-labeled NF-κB probes followed by alkaline phosphatase-conjugated anti-digoxigenin IgG and colorimetric detection (Roche)
	Protein extraction. Western blot of anti-Fn14 (1:1000, Abcam). **N**uclear p65/RelA subunit measured by **ELISA** (Active Motif)

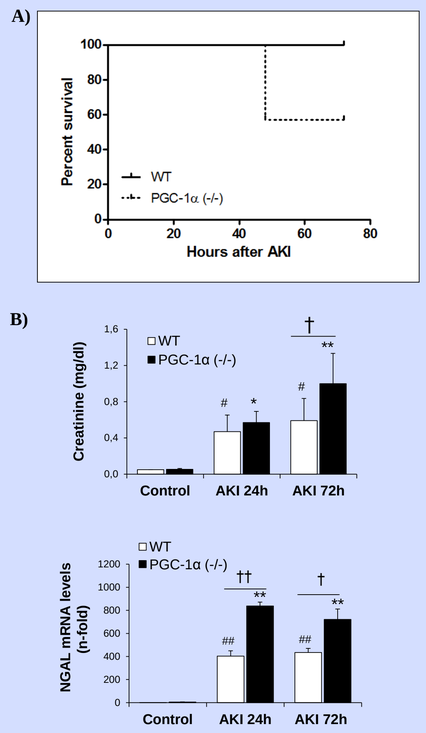
Statistics

Statistical analysis was performed using the SPSS 11.0 statistical software. Results are expressed as mean ± SEM. Significance at the $p<0.05$ level was assessed by nonparametric Mann–Whitney U-test for two groups. # $p<0.05$, ## $p<0.001$ vs control WT; *$p<0.05$, **$p<0.001$ vs control PGC-1α (-/-); † $p<0.05$, †† $p<0.001$ PGC-1α (-/-) vs WT. Pearson correlation was used to assess correlation between two continuous variables.

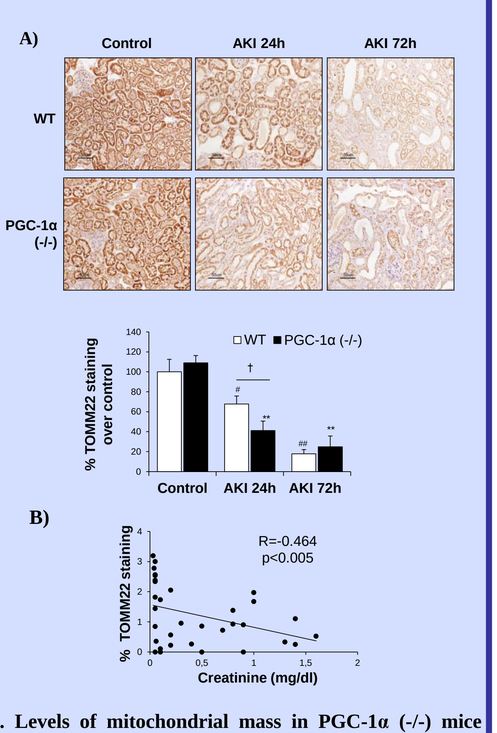
RESULTS



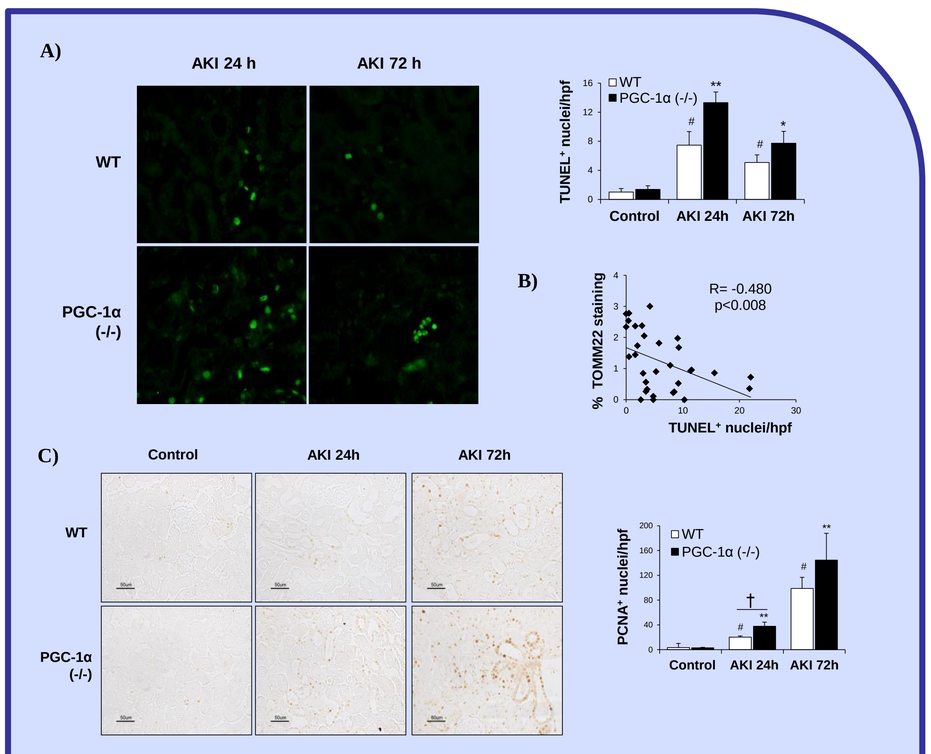
1. Analysis of canonical pathway enrichment using IPA tools shows that PGC-1α and mitochondrial function may play a key role during AKI. **A)** PGC-1α is the most inactive transcriptional regulator in AKI. PGC-1a z-score: -5.66. **B)** Significantly enriched pathways determined using the Benjamini-Hochberg hypothesis (B-H) corrected P value.



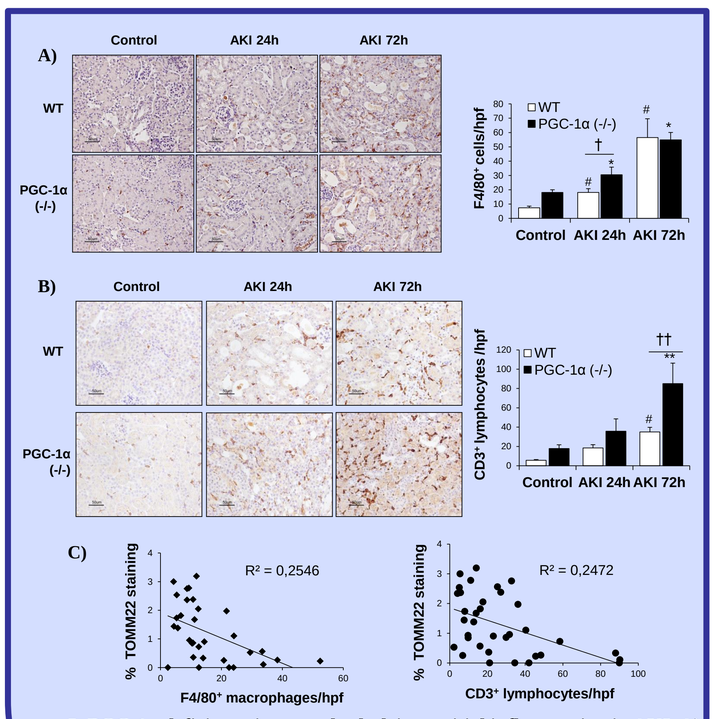
2. Effect of PGC-1α deficiency over survival and renal function during AKI. A) Survival plot. **B)** Renal function assessed by plasma creatinine levels and NGAL mRNA levels.



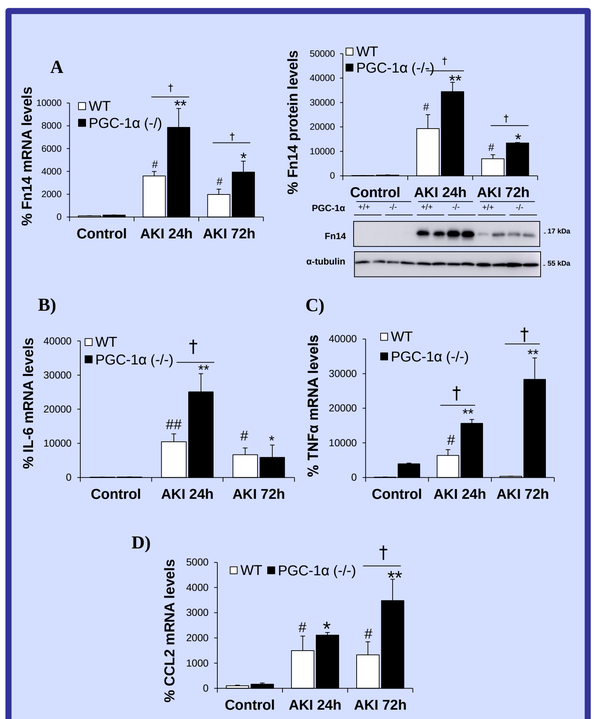
3. Levels of mitochondrial mass in PGC-1α (-/-) mice versus WT mice during AKI. A) Quantification and representative images of TOMM22 staining in renal tissue, which is associated with mitochondrial mass. Original magnification, x200. Scale bars, 50 µm. **B)** Scatter plot showing the significant negative correlation between TOMM22 staining and renal function (n=35).



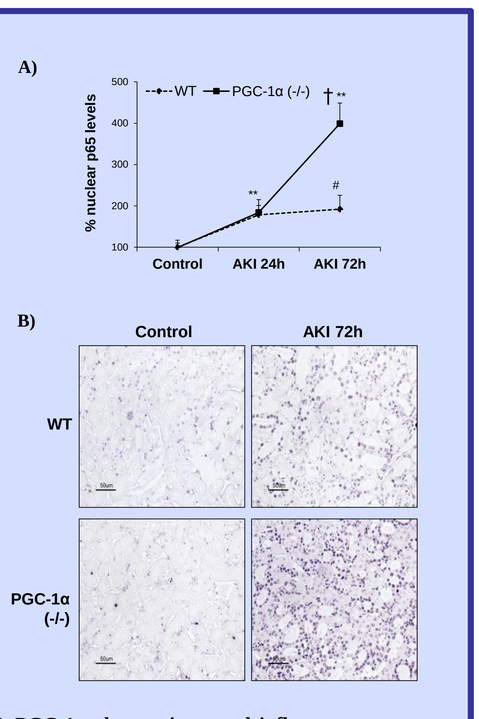
4. PGC-1α deficiency increased the cell death and compensatory proliferation during AKI. A) Quantification and representative images of TUNEL staining to measure cell death. Original magnification, x400. Scale bars, 50 µm. **B)** Scatter plot showing the negative correlation between TOMM22 staining and TUNEL+ nuclei (n=35). **C)** Quantification and representative images of PCNA staining to measure compensatory renal proliferation. Original magnification, x400. Scale bars, 50 µm.



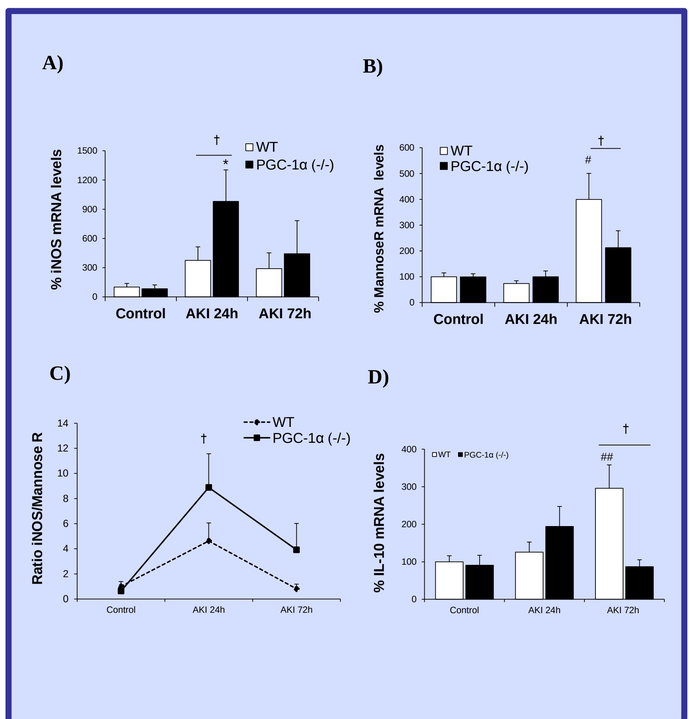
5. PGC-1α deficiency increased tubuleinterstitial inflammation in AKI. A) Quantification and representative images of F4/80 staining in renal tissue. Original magnification, x200. Scale bars, 50 µm. **B)** Quantification and representative images of CD3 staining in renal tissue. Original magnification, x400. Scale bars, 50 µm. **C)** Scatter plot showing significant negative correlation between inflammatory infiltrate and mitochondrial mass (n=35).



6. PGC-1α deficiency increased cytokines and chemokines related with renal injury. A) Fn14 mRNA levels and representative western blot and quantification of Fn14 protein levels. **B-D)** IL-6, TNFα and CCL2 mRNA levels.



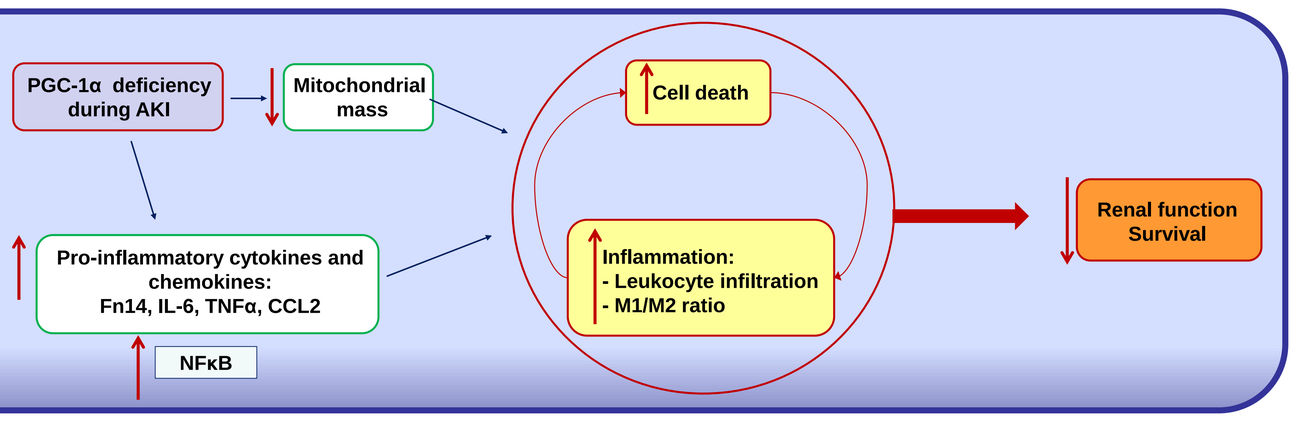
7. PGC-1α absence increased inflammatory response, assessed by an increase in nuclear NFκB p65 levels. A) Nuclear NFκB p65 levels, assessed by ELISA. **B)** Nuclear staining for NFκB p65 measured by Southwestern histochemistry at 72 hours. Original magnification, x200. Scale bars, 50 µm.



8. PGC-1α can modulate macrophage M1-to-M2 transition. A,B) iNOS (M1 marker) and manose receptor (M2 marker) mRNA levels during AKI. **C)** PGC-1α (-/-) mice presented higher ratio M1/M2 (iNOS/Manose R mRNA levels) that WT mice. **D)** mRNA levels of the anti-inflammatory cytokine IL-10.

CONCLUSIONS

PGC-1α is a key driver of the gene expression response in nephrotoxic AKI and PGC-1α deficiency aggravates renal dysfunction and inflammation. These results may be relevant to design therapeutic approaches to upregulate PGC-1α expression during kidney injury



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