Nuclear receptor NR5A2 is involved in the calreticulin gene regulation during renal fibrosis

Eleni Arvaniti[1], Athina Vakrakou[1], Valeria kaltezioti[1], Niki Prakoura[1], Panagiotis Politis[1], Aristidis Charonis[1]

[1]Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Introduction

Renal fibrosis is a common histological finding in many pathologies; however, key signaling pathways and molecular determinants involved in its development are not fully known yet. Previous findings have established a causative role of calreticulin's up-regulation during the development of renal fibrosis while its down-regulation exhibited a protective effect against fibrosis. Therefore, the mechanism of its up-regulation needs to be explored.

Methods

Bioinformatics analyses of the *calreticulin* gene promoter combined with transcriptional assays and in vivo chromatin immunoprecipitation experiments in the Unilateral Ureteric Obstruction (UUO) model of renal fibrosis, indicated that NR5A2 is a critical regulator of calreticulin expression. To confirm this finding, and further study post-translational modifications of NR5A2, real time RT-qPCR, immunohistochemistry and Western blotting experiments were performed.

Results

NR5A2 is up-regulated at both mRNA and protein level during kidney fibrosis in the UUO model. The post-translational modification of SUMOylation was identified as a critical parameter in this phenomenon. SUMOylation was observed to be up-regulated during the development of renal fibrosis. The enzyme Ubc9, critical for this process was also upregulated at mRNA and protein level.

Conclusion

These data establish for the first time a role for NR5A2 and its SUMOylation in a rodent model of renal fibrosis and render NR5A2 a novel target for future anti-fibrotic interventions.

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Results

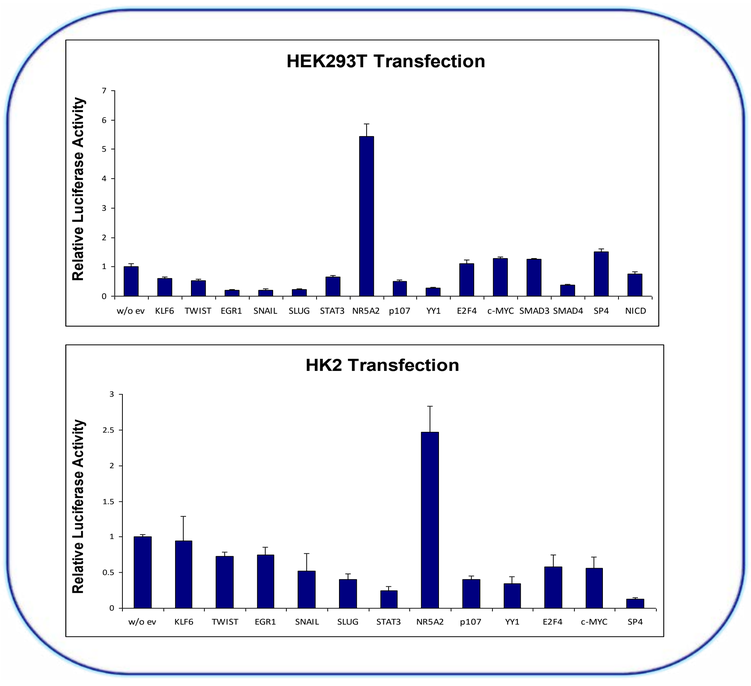

Figure 1. Transcriptional assays in HEK293T and HK2 cell lines, co-transfected with expression vectors and luciferase reporter construct of the *calreticulin* gene promoter. Luciferase relative activity is expressed as fold of change compared to the activity of cells transfected only with the construct of the *calreticulin* gene promoter

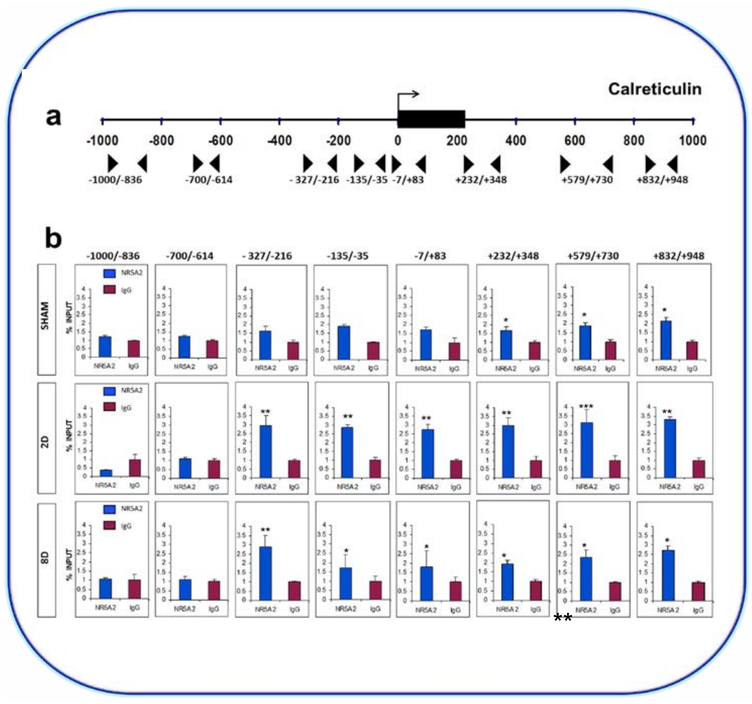

Figure 2. a. Organization of the *calreticulin* gene locus. **b.** ChIP analysis for the binding of NR5A2 to the *calreticulin* promoter in chromatin prepared from sham operated (SHAM); 2 days ligated (2D) and 8 days ligated (8D) mice. * p<0.05; ** p<0.01; *** p<0.005

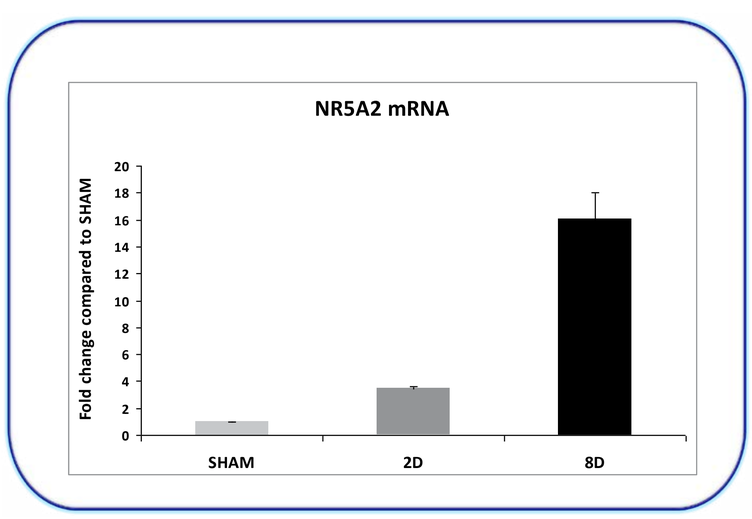

Figure 3. Quantification of NR5A2 expression by Real-time PCR analysis. * p<0.05; ** p<0.01

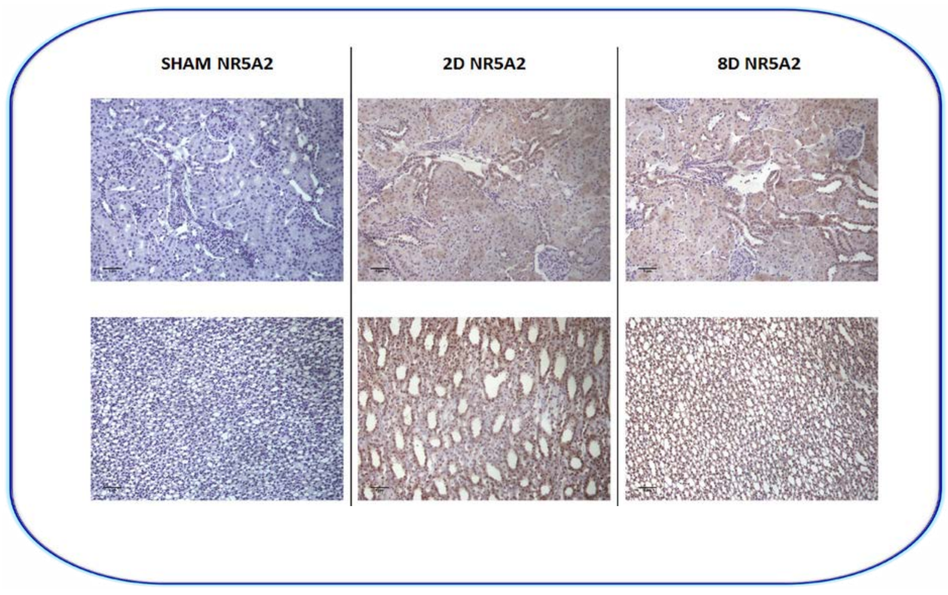

Figure 4. Localization of NR5A2 in kidney sections of the UUO model. Magnification x200.

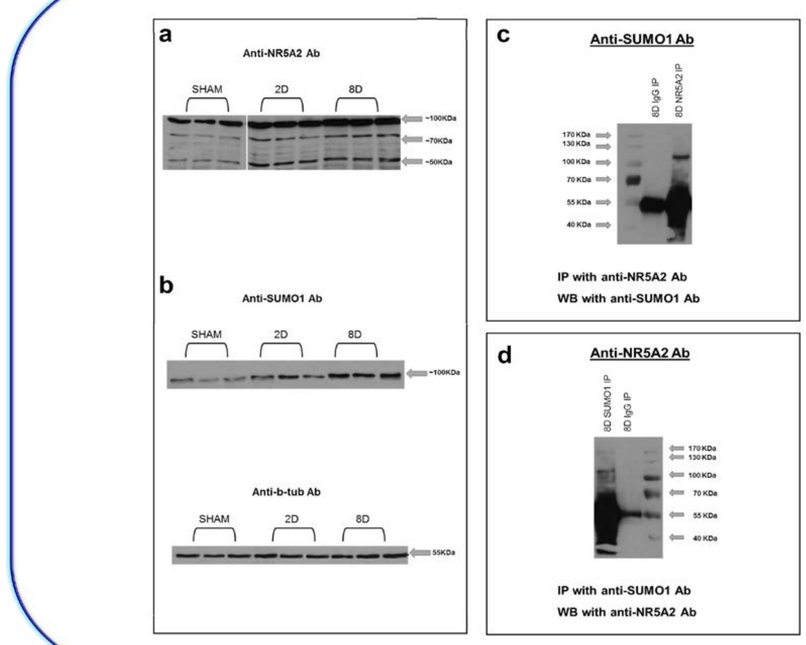

Figure 5. a-b. Western blot analysis of NR5A2 and SUMO1 in SHAM; 2D and 8D mice. **c-d.** Immunoprecipitation experiments

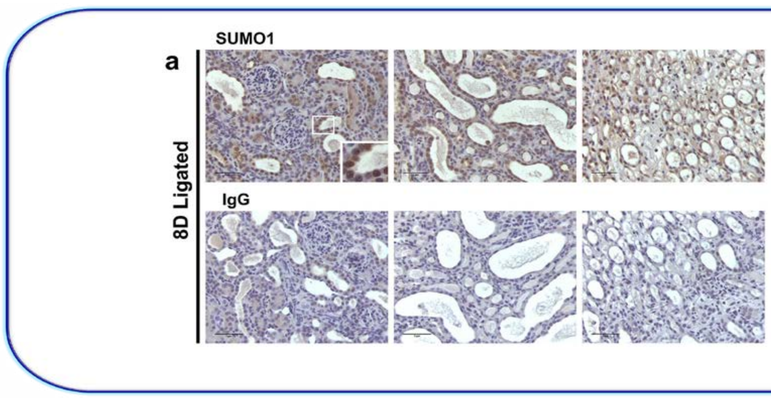

Figure 6. Localization of SUMO1 in 8D kidney sections taken from 8D ligated mice. Magnification x400.

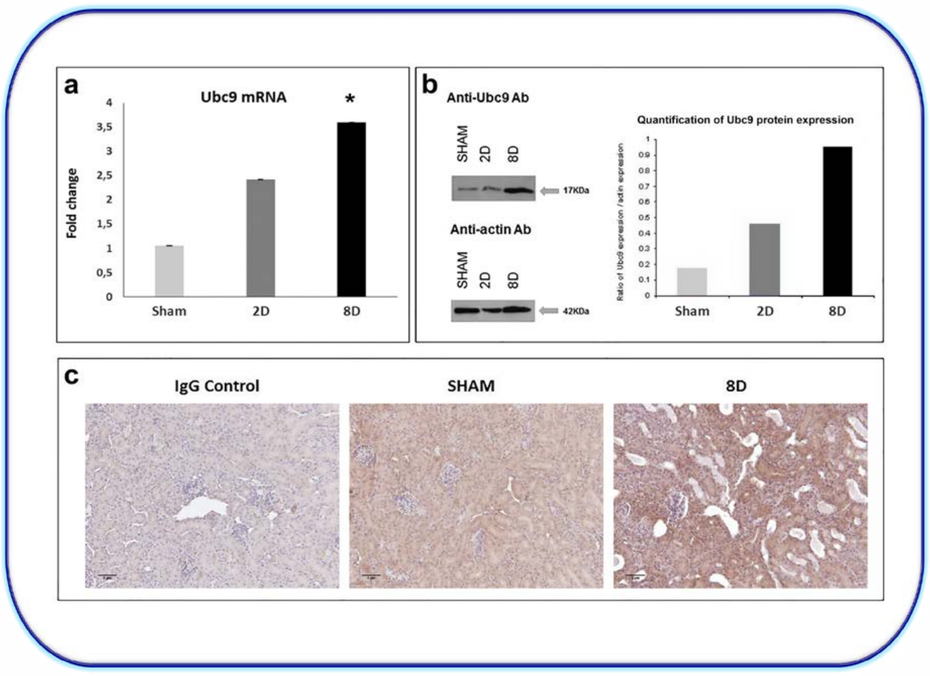

Figure 7. a. Quantification of Ubc9 expression by Real-time PCR analysis. * p<0.05 **b.** Western blot analysis of Ubc9 in SHAM; 2D and 8D mice. **c.** Localization of Ubc9 in kidney sections from SHAM and 8D mice. Magnification x200.

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