ADIPONECTIN IS EXPRESSED AND SECRETED BY TUBULAR EPITHELIAL RENAL CELLS

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CENTRO DI RICERCA RENE E TRAPIANTO

BACKGROUND AND AIM

Adiponectin (ADPN) is an adipokine with anti-atherogenic, anti-inflammatory and insulin-sensitizing properties (1). To date is reported that the adipocyte is the predominant cell type responsible for secretion of ADPN and that the chronic inflammatory status characterizing obese patients is responsible for a reduction of ADPN circulating levels (2). *In vivo* studies demonstrated that the hypoadiponectinemia induces microalbuminuria through a direct alteration of membrane permeability mediated by podocytes dysfunction (3). However clinical studies demonstrated that patients with overt proteinuria had higher circulating ADPN levels compared to normoalbuminuric controls. Unexpectedly, similar ADPN levels were observed in obese proteinuric patients. To date, despite all these evidences, the mechanisms linking overt proteinuria and hyperadiponectinemia are not yet clarified.
The AIM of our study was to investigate whether epithelial tubular renal cells express and secrete adiponectin and, principally, whether renal cells in basal conditions and upon an inflammatory stimulus secrete this adipokine contributing to ADPN circulating levels.

MATERIALS AND METHODS

Immortalized human proximal tubular epithelial cells, HK-2, (ATCC) were cultured in Keratinocyte Serum Free Media (K-SFM) supplemented with bovine pituitary extract (BPE 0,05mg/ml) and human recombinant epidermal growth factor (EGF 5 ng/ml) (Invitrogen) at 37°C in 5% CO2. Human subcutaneous preadipocytes, purchased by Zen-Bio, were obtained from adipose tissue of healthy non-diabetic donor 35 years old with BMI = 23.5. The undifferentiated preadipocytes were inoculated into the appropriate culture plates and subsequently differentiated using Ze-Bio's differentiation (DM-2) and adipocyte (AM-1) media. Mature adipocytes were used as positive control. In HK-2 cells and adipocytes ADPN, AdipoR1 and Adipor2 mRNA contents were evaluated by real time RT-PCR-assay and PCR assay, respectively, while their protein expression levels were assessed by Western blot analysis. In HK-2 cells ADPN localization was investigated by immunofluorescence assay. Moreover, renal ADPN distribution was assessed by immunohistochemical analysis of kidney biopsies from healthy subject and from two patients affected by rapidly progressive and membranous glomerulonephrytis respectively. Finally, by ELISA assay (R&D System), we measured ADPN concentrations in culture media of HK-2 cells treated with lipopolysaccharide (LPS) 10 µg/ml for 6,12 and 24 hours. Prior to treatments HK-2 were starved in serum free medium (SFM) for 12 hours.

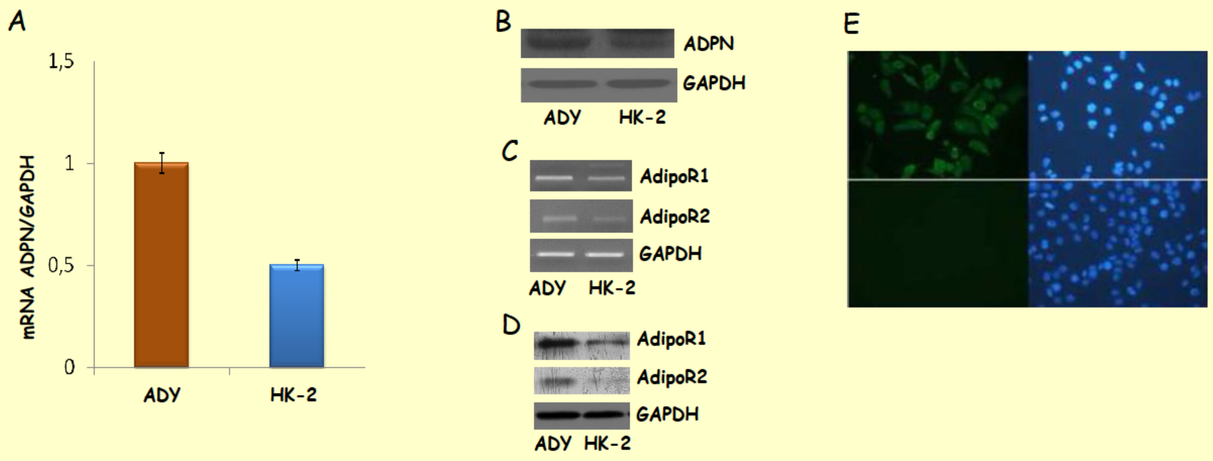

Fig 1 ADPN and its receptors expression in HK-2 cells GAPDH was used as internal control and Adipocytes (ADY) as positive control. All the experiments were executed in triplicate and one of three similar experiments is presented. (**A**) ADPN RNA content evaluated by real-time RT-PCR assay in HK-2. (**B**) Immunoblot of ADPN in HK-2 cells. (**C**) AdipoR1 and AdipoR2 mRNA expression evaluated by RT-PCR in HK-2 cells. (**D**) Immunoblots of AdipoR1 and AdipoR2 in HK-2 cells (**E**) HK-2 cells were cultured and immunostained for ADPN (green fluorescence): nuclei were counterstained with DAPI. (Negative) cells were incubated replacing the anti-ADPN antibody by normal mouse IgG utilized as negative control. Original magnification: x40.

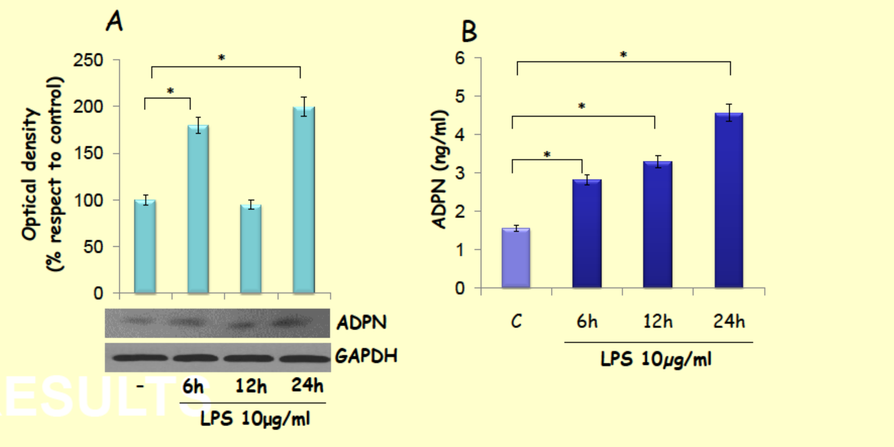

Fig 2. ADPN expression and secretion upon LPS exposure HK-2 cells were starved in SFM for 24 h and then treated with vehicle (-) or with LPS 10µg/ml for 6, 12 and 24 h as indicated. (**A**) Immunoblot of ADPN in HK-2 cells. GAPDH was using as loading. The upper panel shows the means ± SD of three independent experiments, each performed in triplicate and expressed as percentages of the control which was assumed to be 100%. (**B**) ADPN concentration (ng/ml) in cellular surnatant collected by HK-2 cells in basal condition and after treatment with LPS 10µg/ml for 6, 12 and 24 h as indicated. * p<0.05.

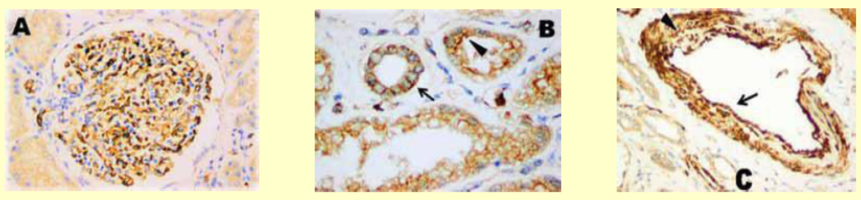

Fig. 3 ADPN in healthy human kidney (**A**) ADPN expression in a normal glomerulus at higher magnification. (**B**) ADPN in tubular epithelial cells (arrow) and along the brush border (arrowhead). (**C**) ADPN on the endothelium (arrow) and smooth muscle cells (arrowhead) of intra-renal arteries/arterioles.

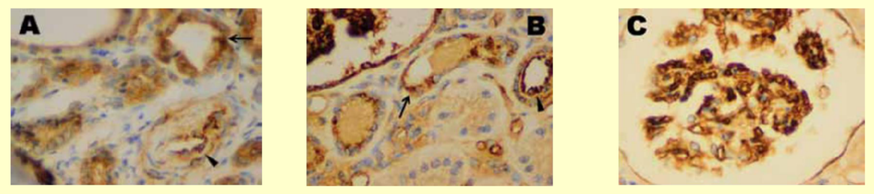

Fig. 4 ADPN in human glomerulonephritis (**A**) ADPN expression in endothelium, smooth muscle cells and tubular epithelial cells in rapidly progressive glomerulonephritis. ADPN expression in endothelium, smooth muscle cells, tubular epithelial cells (**B**) and glomerulus (**C**) in membranous glomerulonephritis.

RESULTS

Our analyses revealed that HK-2 cells express ADPN both in terms of mRNA and protein. These results were confirmed by the observed cytoplasmatic HK-2 intense immunoreactivity for ADPN antibody and by immunohistochemical analysis showing a diffuse ADPN distribution in normal kidney tissue. We also confirmed that HK-2 cells express both best-characterized receptors for ADPN, adipoR1 and adipoR2 although, the results revealed that adipoR1 is the predominant isoform. Furthermore we observed that tubular cells secrete ADPN in basal condition and, more interestingly, this secretion significantly increases ($p < 0.05$) upon LPS treatment in a time dependent manner. Finally, immunohistochemical analysis of kidney biopsies obtained from patients affected by membranous and rapidly progressive glomerulonephritis showed a similar pattern of ADPN staining observed in healthy control.

CONCLUSIONS

Our study demonstrates, for the first time, that tubular renal cells express and secretes ADPN, which concentration increases upon inflammatory stimulus. These results suggest that in renal inflammatory diseases, tubular cells may contribute to the increasing ADPN circulating levels, triggering a feedback response in order to self-mitigate the inflammatory process.

REFERENCES

1 Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem. 1995
2 Hoffstedt J, Arvidsson E, Sjölin E, Wåhlén K, Arner P. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. J Clin Endocrinol Metab. 2004
3 Sharma K, Ramachandrarao S, Qiu G, et al. Adiponectin regulates albuminuria and podocyte function in mice. J Clin Invest. 2008

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