ical variables and the renal AT_1 receptor and ATRAP immunostaining revealed ATRAP protein expression to be significantly and positively correlated with eGFR. The other parameters of renal function, e.g., urinary protein and serum creatinine, were not significantly associated with ATRAP protein expression (Fig. 7). The eGFR values may reflect disease (IgA nephropathy)-induced deterioration of renal function. Whether eGFR or renal ATRAP protein expression is the cause remains to be determined. It seems that the eGFR status should be the cause of changes in renal ATRAP protein expression; however, this is difficult to address, and additional studies, i.e., repeated biopsies and/or prospective follow-up, are needed.

Furthermore, since we examined the expression and distribution of ATRAP mRNA and protein in normal human renal tissue sections from one patient with renal cell carcinoma without other obvious chronic kidney disease, a limitation of the present study is that it did not examine a possible association between AT_1 receptor and ATRAP in the normal human kidney. Another limitation is the lack of immunofluorescent colocalization analysis with double staining of ATRAP and the AT_1 receptor using a multiple fluorolabeling method and confocal laser microscopy. Nevertheless, these results suggest that the decrease in eGFR, as a strong cardiovascular risk factor for "cardio-renal syndrome," might influence renal ATRAP expression and thereby play a critical role, presumably, affecting AT_1 receptor signaling in renal tissues.

Finally, to investigate the function of tubular ATRAP in vitro, we used an immortalized cell line (mDCT cells). These cells have been shown to have a polarized tight junction epithelium with morphological and functional features retained from parental cells, and they have been previously characterized at the molecular level with respect to their responsiveness to various hormones and agents (8, 9). In the present study, ATRAP was abundantly expressed and widely distributed along the renal tubules, including the DCT cells. Furthermore, in all tubular cells, including DCT cells, ATRAP protein was colocalized with AT_1 receptor protein, based on immunohistochemical analysis. Since previous studies showed that ATRAP inhibited ANG II-induced pathological responses of cardiovascular cells by promoting a constitutive internalization of the AT_1 receptor (24, 32), we examined whether renal tubular ATRAP antagonizes the pathological activation of the tubular AT_1 receptor using mDCT cells. The results showed that the overexpression of ATRAP suppresses the AT_1 receptor-mediated activation of TGF-β production in response to ANG II stimulation, thereby suggesting that tubular ATRAP is an endogenous suppressor of the activation of tubular AT_1 receptor signaling.

In summary, the results of the present study demonstrate the abundant expression of ATRAP mRNA and protein and their distribution in the human kidney. ATRAP is broadly distributed along the nephron, with a substantial colocalization of ATRAP and the AT_1 receptor. The results using human needle renal biopsy specimens of IgA nephropathy show a significant relationship between renal ATRAP and AT_1 receptor protein levels, and renal ATRAP protein expression appears to be influenced by renal functional status. Furthermore, the findings obtained by in vitro experiments using renal distal tubular cells also showed the functional significance of renal tubular AT_1 receptor signaling, as well as the antagonistic effect of tubular ATRAP on this signaling. These findings suggest that in addition to the AT_1 receptor, ATRAP, a newly emerging component of the renin-angiotensin system, is likely to play a role in balancing the renal renin-angiotensin system by counterregulatory effects, which in turn may be confounded by the presence of chronic kidney disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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