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Therapeutic Strategy against Salt Hypertension and Renal Aging via Novel Dual Actions of Receptor-binding Molecule

Kouichi Tamura[1], Hiromichi Wakui, Akio Yamashita[2], Kengo Azushima[1], Kazushi Uneda[1], Ryu Kobayashi

[1]Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, [2] Department of Molecular Biology, Yokohama City University Graduate School of Medicine

Summary

Background:

Angiotensin II type 1 receptor (AT1R)-associated protein (ATRAP), which was originally identified as a molecule that binds to AT1R, is highly expressed in the kidney. We have shown that ATRAP functions as an endogenous inhibitor that suppresses AT1R hyperactivation at local tissue sites. In addition, we recently demonstrated that ATRAP deficiency exacerbates ageing-associated renal function decline and tubulointerstitial fibrosis using systemic ATRAP knockout mice. As a key mechanism, renal SIRT1 expression was significantly decreased in the aged ATRAP-knockout mice compared to the aged wild-type mice, possibly in an angiotensin-independent manner. The present study was performed to investigate the functional role of ATRAP in proximal tubules using proximal tubule-specific ATRAP knockout (PT-KO) mice and aclonal immortalised human renal proximal tubule epithelial cell line (ciRPTEC).

Methods:

We created proximal tubule-specific ATRAP knockout (PT-KO) mice using the Cre/loxP system with Pepck-Cre, and examined a functional role of proximal tubule ATRAP in angiotensin-mediated hypertension. Normal human RPTEC cells were immortalised by infection with lentivirus expressing hTERT and short hairpin RNA (shRNA) targeting p16 (plenti6_TERT_sh-p16). Then, we cloned the immortalised RPTEC and characterised the cells based on the expression of two proximal tubule markers, SGLT2 and DPP4. ATRAP knockdown and knockout tests were performed using ciRPTEC.

Results:

The BP of PT-KO mice was comparable with that of wild type (WT) mice at baseline. Moreover, the BP was significantly and similarly increased in response to 2 weeks of Ang II infusion in both PT-KO and WT mic. In addition, cumulative sodium balance during Ang II infusion was comparable for PT-KO and WT mice. In ciRPTEC, ATRAP-knockdown significantly reduced the SIRT1 protein expression level in the steady state. On the other hand, there was no significant difference in the expression level of SIRT1 mRNA by ATRAP-knockdown. Furthermore, in ATRAP-knockout cells compared to control cells, SIRT1 protein expression was significantly reduced in a serum starvation-dependent manner.

Conclusion:

We demonstrated that proximal tubule-specific down-regulation of ATRAP did not affect the Ang II-mediated hypertension *in vivo*. The results of *in vitro* study indicated that ATRAP may be one of the molecules involved in regulating the abundance of SIRT1 protein but not SIRT1 mRNA independent of blocking of AT1R signaling.

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