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Calcium-mediated Calpain elevation induces tubulointerstitial fibrosis in Nephronophthisis

Dantong Li[1], Jinglan Zhang[2], Zhiming Ye[1], Liangzhong Sun[2]
[1]Department of Internal Medicine-Nephrology, Guangdong Provincial People's Hospital, China
[2]Department of Pediatrics, Nanfang Hospital, Southern Medical University, China

Objectives : Nephronophthisis (NPH) is the most common genetic disease leading to renal failure in children. Mutations in Nphp1 account for the majority of NPH cases, and renal tubulointerstitial fibrosis is one of the most important pathological features. However, the molecular mechanisms of tubulointerstitial fibrosis remained unknown.
Methods : RNA sequencing was carried out in Nphp1-targeted cells and mice kidneys to explore the mechanisms of renal tubulointerstitial fibrosis. Calpain (CAPN) was found to be upregulated in both models. We verified the upregulation of CAPN in NPH models and a CAPN inhibitor was used to explore its role in tubulointerstitial fibrosis. To further clarify the mechanism of CAPN-mediated tubulointerstitial fibrosis in NPH models, we used gene set enrichment analysis and found that the autophagy-lysosomal pathway was inhibited. We detected autophagy activities in NPH models with CAPN inhibition by immunofluorescence, lysotracker and transmission electron microscopy (TEM). Calpain is calcium-dependent in previous studies. To discover how the calpain is regulated in NPH models, we detected the calcium concentration by Fluo-4 AM. Furthermore, calcium-containing and calcium-free cell culture media were utilized to explore the effect of calcium concentration on CAPN expression.
Results : CAPN was verified to be upregulated in both Nphp1-targeted HK2 cells and mice kidneys. We also discovered impaired autophagic flux in NPH models. Furthermore, the impaired autophagic flux induced tubulointerstitial fibrosis in NPH models. However, the autophagic flux was improved and the status of tubulointerstitial fibrosis was ameliorated with CAPN inhibition. An increase of calcium concentration in NPH models was discovered. When using calcium-free cell culture media, the expression of CAPN was decreased in Nphp1-targeted HK2 cells.
Conclusions : CAPN-mediated autolysosomal pathway disorder may be an important cause of tubulointerstitial fibrosis in NPH. The upregulation of CAPN may be induced by increased calcium concentration in NPH models. CAPN may be a new target for diagnosis and treatment of NPH.

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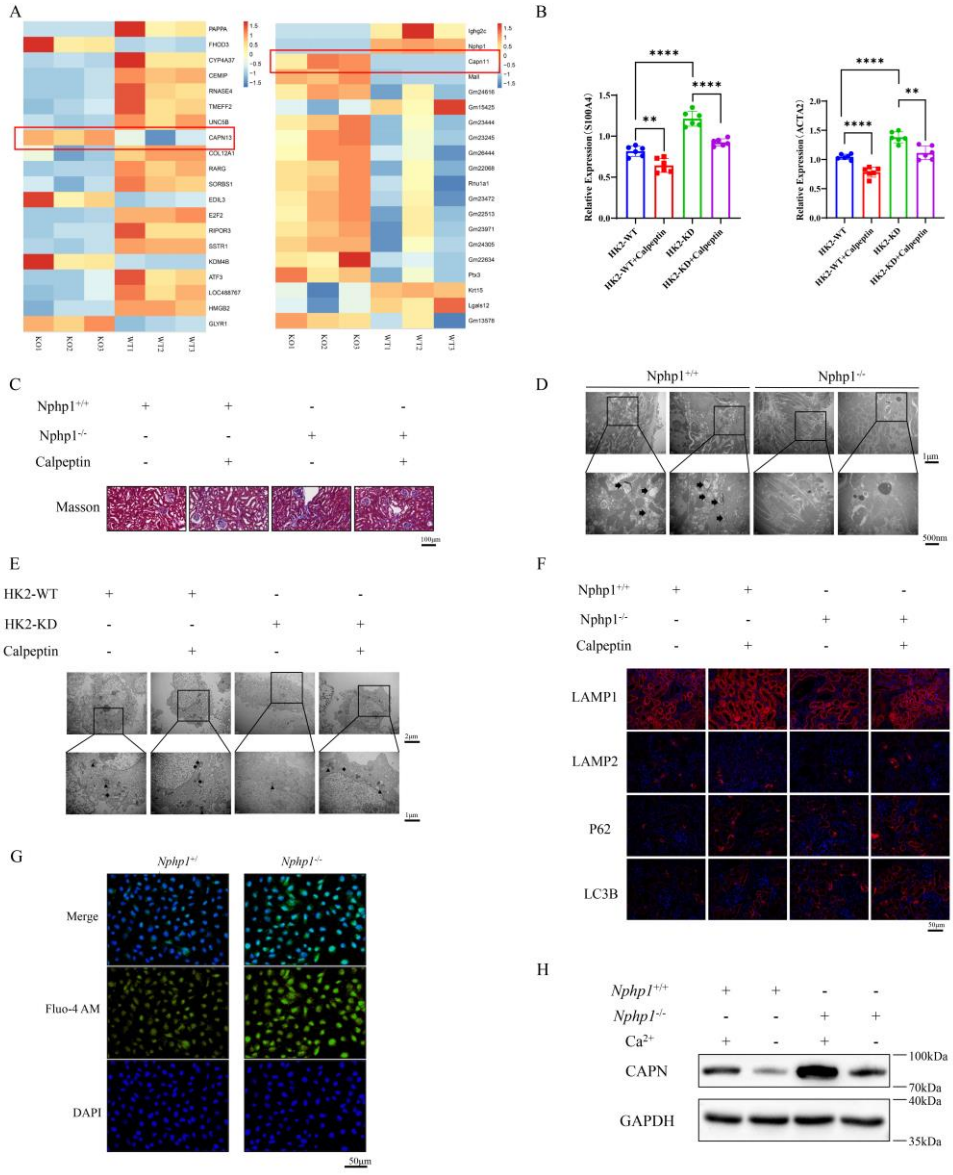
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