Modifying TRPM7 Function by Synthetic Small Interfering RNA and CHBP in Mouse Renal Epithelial Cells

Aifen Liu[1], Jing Wu[2] , Cheng Yang[3] , Li Yuan[2], Hui Wang[2], Yuanyuan Wu[1], Yufang Zhang[1] , Yaqiu Long[4] , Tongyu Zhu[3] , Yaping Fan[2] and Bin Yang[1,2,5]

[1]Basic Medical Research Centre, Nantong University; [2]Nephrology, Affiliated Hospital of Nantong University; [3]Pathology, Nantong University, China; [4]CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China; [5]Infection, Immunity and Inflammation, University of Leicester, UK

Introduction

Transient receptor potential melastatin 7 (TRPM7) is a nonselective cation channel, and its activation leads to calcium overload and subsequent injuries. Our previously revealed that TRPM7 expression was increased in renal related ischemia reperfusion injury (IRI) *in vitro* and *in vivo* models, which was reversed by a novel cyclic helix B peptide (CHBP), erythropoietin derivative. Synthetic small interfering RNA (siRNA) transiently silenced target genes posttranscriptionally in renal IRI.

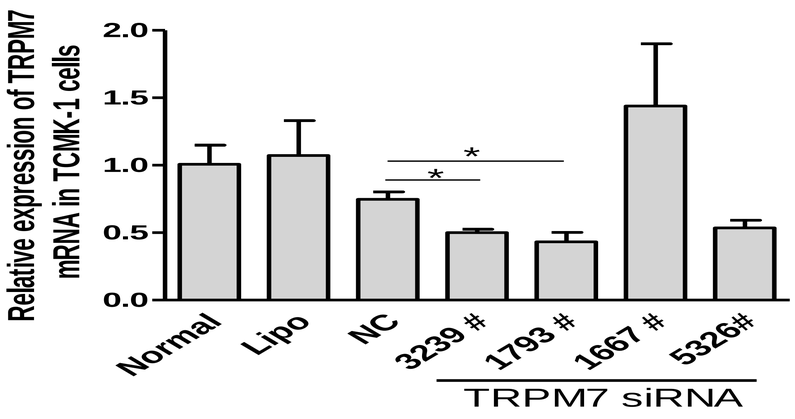
Aims

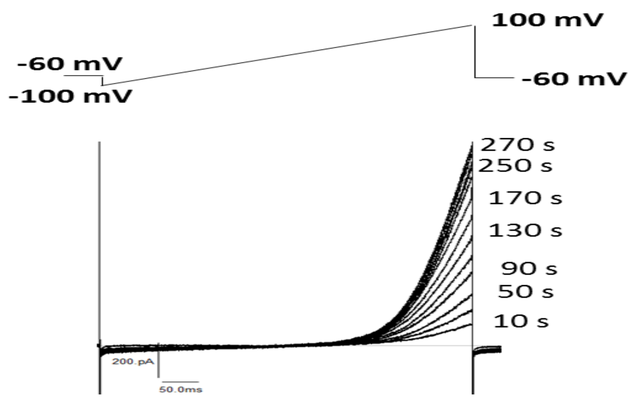
Here, we further investigated the effects of modifying TRPM7 by siRNA or CHBP on its down stream function in mouse renal epithelial TCMK-1 cells.

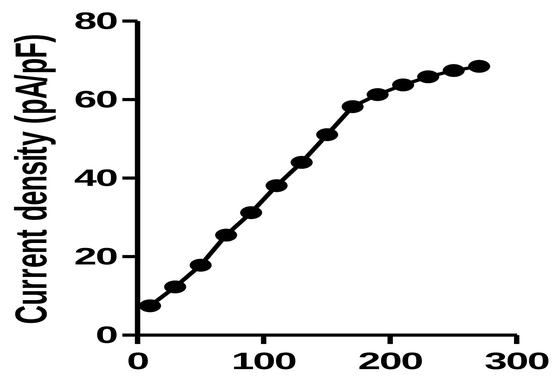
Methods

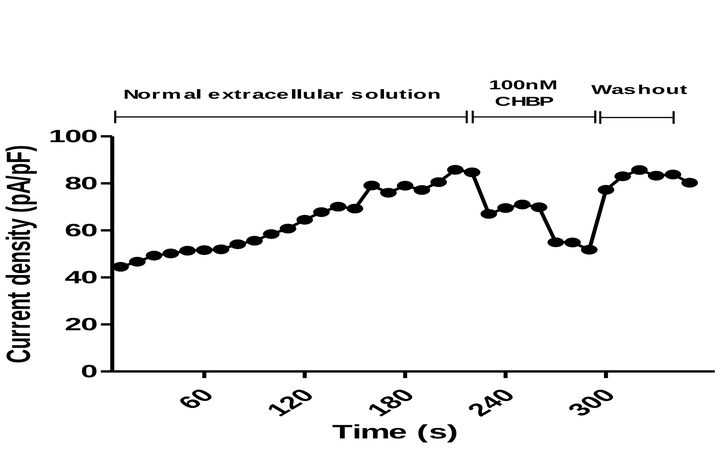
After TCMK-1 cells were transfected with synthetic TRPM7 siRNA using a cationic lipid-based transfection regent lipofectamine 2000, the expression of TRPM7 mRNA expression was detected to select the most effective sequence of TRPM7 siRNA. Using whole-cell patch-clamping, TRPM7 currents were first recorded in TCMK-1 cells, and then the regulation of TRPM7-like currents by TRPM7 siRNA or CHBP was also investigated.

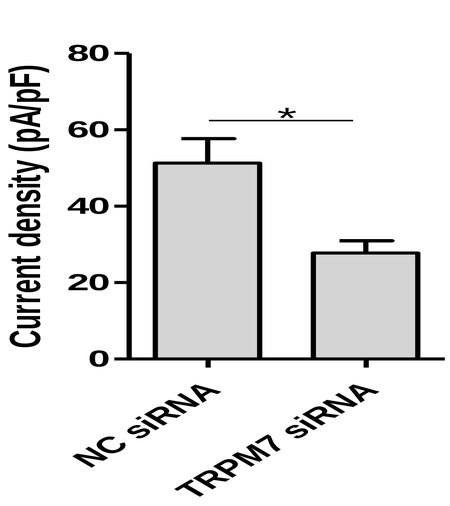
Results

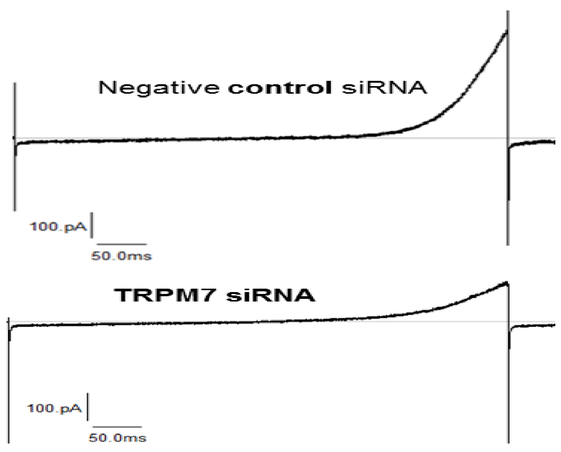


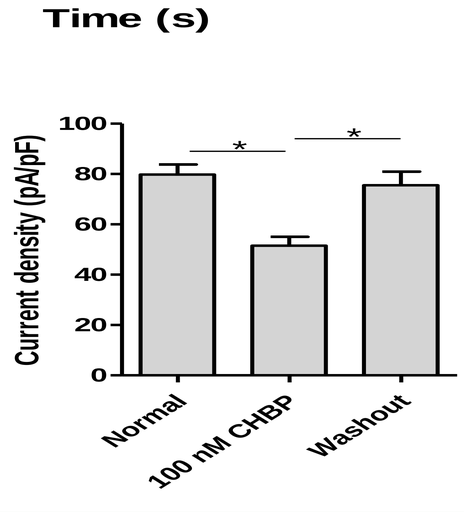












Summary

- In the TCMK-1 cells transfected with TRPM7 siRNA with sequence number 3239, 1793 and 5326, the expression of TRPM7 mRNA was respectively reduced by 33%, 42% and 28% compared to the cells treated by the negative control siRNA for 24 h.
- inward and outward TRPM7 currents were time-dependently increased using pipette filled with internal Mg2+-free solution, and the TRPM7-like current was successfully identified in TCMK-1 cells.
- TRPM7 siRNA 1793 sequence at 40 nM for 48 h caused a noticeable reduction of outward TRPM7 current in comparison to cells treated with the negative control siRNA.
- CHBP treatment at 100 nmol/L also inhibited the TRPM7-like current in TCMK-1 cells.

Conclusions

Synthetic siRNA targeting TRPM7 and CHBP both decreased TRPM7-like currents, suggesting a potential renoprotection that might associate with the inhabitation of calcium overload. However, the function and mechanism of TRPM7 in renal IRI, as well as the renoprotection of CHBP, are worthy to be further investigated.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (81130569 to AFL & 81570677 to BY), Nantong University and the University of Leicester.