**Phosphodiesterase 4D promotes angiotensin II-induced hypertension in mice via smooth muscle cell contraction**

Tian-Fei Fan1\*, Yang-Feng Hou1\*, Wei-Peng Ge1, Tian-Hui Fan1, Wen-Jun Guo1, Xiao-Min Song1, Ran Gao1#, Jing Wang1#

1 State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences; Department of Pathophysiology, School of Basic Medicine, Peking Union Medical College, Beijing 100005, China.

# Co-corresponding author:

Dr Jing Wang, State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences; Department of Pathophysiology, School of Basic Medicine, Peking Union Medical College, Beijing 100005, China. E-mail: wangjing@ibms.pumc.edu.cn.

Dr Ran Gao, State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences; Department of Pathophysiology, School of Basic Medicine, Peking Union Medical College, Beijing 100005, China. E-mail: ggrr1991@outlook.com.

\* These authors contributed equally.

**Supplementary Figures**



**Supplementary Figure 1. Blood pressure (BP) of angiotensin (Ang) II induced hypertensive mouse model**. a) Systolic and b) diastolic BPs were measured at baseline and after Ang II infusion for 14 days. Hypertensive mice were induced by Ang II infusion (490 ng/kg/min) for 14 days; controls were infused with saline (n = 4 in control group, n = 8 in Ang II group). Data are expressed as mean ± SEM. \*\*\**P* < 0.001.



**Supplementary Figure 2. Identification of endothelial cell (EC)-specific knockout phosphodiesterase (PDE) 4D (PDE4DEC-/-) mice and smooth muscle cell (SMC)-specific knockout PDE4D (PDE4DSMC-/-) mice**. a) PDE4DEC-/- and PDE4DSMC-/- mice were generated via Cre-LoxP recombinase system using CRISPR/Cas9 technology. Exon 10 was deleted via Tek-Cre or Tagln-Cre–mediated recombination. b) Genotyping was detected by tail preparation of 2-week old PDE4Dflox/flox and PDE4DEC-/- mice. c) Representative western blot showing PDE4D expression in the aorta from PDE4Dflox/flox and PDE4DEC-/- mice (n = 2 in each group). d) Genotyping was detected from the tails of 2-week old PDE4Dflox/flox and PDE4DSMC-/- mice. e) Representative western blot showing PDE4D expression in the aorta from PDE4Dflox/flox and PDE4DSMC-/- mice (n = 2 in each group).



**Supplementary Figure 3. mRNA and protein expression of PDE4D after transfection with PDE4D small interfering RNA (siRNA) in rat aorta smooth muscle cells (RASMCs)**. a) Representative western blot showing PDE4D expression in RASMCs treated with control siRNA or PDE4D siRNA (200 nM, 48 hrs). b) Quantification of PDE4D expression normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein (fold change vs. controls). c) Real-time polymerase chain reaction (RT-PCR) to measure *Pde4d* mRNA expression in RASMCs with control siRNA or PDE4D siRNA for 48 hrs (200 nM) (fold change vs. controls). Data are expressed as mean ± SEM; n = 3 per group. \**P* < 0.05, \*\**P* < 0.01.

**Supplementary Table**

**Supplementary Table 1. Primer sequences**

PDE: phosphodiesterase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Ko: knockout.

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| --- | --- | --- | --- |
| Genes | Species | Forward-Primer | Reverse-Primer |
| PDE4A | Mouse | AAAGCTGGTACACACCGGAAG | CAGGCCCCATTTGCTCAAGT |
| PDE4B | Mouse | CAACGCCAGACACTCAGGAA | AGAACACCGGAGCTTGTCAC |
| PDE4C | Mouse | GGTGTGATCCTGAGACGGTT | TTTCGAGGTCAAAGCTGCTC |
| PDE4D | Mouse | CTCTCGGAGCAAAAGTGCCT | GATTCGCTTCGCAAGTCTGC |
| GAPDH | Mouse | TCAACGACCACTTTGTCAAGCTCA | GCTGGTGGTCCAGGGGTCTTACT |
| PDE4D | Rat | CCATGTGCAACCAACCATCC | GGTGAGCTCCCGATTAAGCA |
| GAPDH | Rat | GGTGCTGAGTATGTCGTGGA | ACTGTGGTCATGAGCCCTTC |
| PDE4D flox | Mouse | TATTGCCCAGGAAACAGTAACA | GACATCGGCCTTTTCTTCTTCTCC |
| PDE4D Ko | Mouse | TTGCCCAGGAAACAGTAACAGAAG | AAACAAGCCACCAAACAGCAACAA |
| Tagln-Cre | Mouse | TCGATGCAACGAGTGATGAG | TCCATGAGTGAACGAACCTG |
| Tek-Cre | Mouse | TCGATGCAACGAGTGATGAG | TCCATGAGTGAACGAACCTG |
| Positive control | Mouse | CAAATGTTGCTTGTCTGGTG | GTCAGTCGAGTGCACAGTTT |