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ANGIOTENSIN II PROMOTES DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS TO VASCULAR SMOOTH MUSCLE CELLS THROUGH PI3-KINASE SIGNALLING PATHWAY AND NF-κB

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Xiaoye Zheng, Yutao Wu, Qishan Chen, Yijiang Zhou, Li Zhang, Jianhua Zhu.
Department of Cardiology, the First Affiliated Hospital, School of Medicine, Zhejiang University

Objectives Embryonic stem cells (ES cells), the pluripotent derivatives of inner cell mass of blastocysts, have the capacity for

unlimited growth, self-renewal and differentiate into all types of mature tissue cells. Angiotensin II (Ang II), the most important effector peptide of the renin–angiotensin system, is also an angiogenesis factor. This study attempted to explore the potential impact of Ang II on ES cells differentiation and the underlying mechanisms.

Methods Mouse embryonic stem cells (ES-D3) were plated on collagen IV-coated dishes/flasks in differentiation medium for 2, 4, 6, 8 days, and a panel of smooth muscle specific markers, including smooth muscle a-actin (SMA), calponin, SM22, mouth muscle myosin heavy chain (SM-MHC) were detected by qRT-PCR and western blot. When the 4-day predifferentiated ES cells were pretreated with different concentrations (10^{-10}–10^{-6} mol/l) of Ang II for 48 h, the mRNA, protein expression and immunofluorescent staining of smooth muscle specific markers were also detected. Besides, the 4-day predifferentiated ES cells were pre-incubated with different inhibitors, such as Ang II type 1 (AT1) receptor antagonist losartan (25 μmol/l, 50 μmol/l), Ang II type 2 (AT2) receptor antagonist PD123319 (1 μmol/l, 10 μmol/l), NF-κB inhibitor BAY11-7082 (5 μmol/l) and phosphoinositide-3 kinase (PI3K) inhibitor LY294002 (10 μmol/l), and the related expression of smooth muscle specific markers and phosphor-Akt were detected by western blot. The SMA and SM22 promoter activity on different concentrations of Ang II (10^{-10}–10^{-6} mol/l) were detected by Luciferase reporter assay, and transcription factors c-Jun, NF-κB p50, NF-κB p65 were accordingly analysed by qRT-PCR. Finally, the protein expression of two proinflammatory factors, TNF-a and c-fos during Ang II (10^{-9} mol/l) stimulation were detected by western blot.

Results We have successfully induced the differentiation of ES cells into vascular smooth muscle cells (SMCs) on collagen IV, and a panel of SMC-specific genes was significantly and consistently upregulated. Interestingly, incubation of ES cells with Ang II further promote SMC differentiation from ES cells, which was abolished by prior treatment with Ang II type 1 (AT1) receptor antagonist losartan ($p<0.01$), but not Ang II type 2 (AT2) receptor antagonist PD123319 ($p>0.05$). Ang II can directly promote the smooth muscle specific markers expression at the transcription level, as revealed that Ang II increased SMA-reporter activity, with the discernable effect observed at 10^{-8} mol/l and 10^{-9} mol/l ($p<0.05$). Moreover, we found that, in parallel with SMC specific-markers induction, the expression levels of phosphor-Akt and NF-KappaB (NF-κB) p50 were upregulated by Ang II, with the maximum stimulation observed at 10^{-9}mol/l ($p<0.01$). Importantly, addition of phosphoinositide-3 kinase (PI3K) inhibitor LY294002 led to a marked inhibition of Ang II induced VSMC specific markers, phosphor-Akt and NF-κB p50 expression ($p<0.01$). Furthermore, NF-κB inhibitor BAY11-7082 can inhibit Ang II induced expression of SMC specific markers ($p<0.01$). However, the protein expression of two proinflammatory factors TNF-a and c-fos were not changed much ($p>0.05$).

Conclusions Thus, we demonstrate for the first time that Ang II plays a promotive role in the stage of ES cells differentiation to SMCs through AT1 receptor. We further confirmed that PI3K/Akt signalling pathway and NF-κB plays a key role in this process. Besides, Ang II can directly promote the smooth muscle specific markers expression at the transcription level, through stimulating many transcription factors especially transcription factor NF-κB p50, which may has the close relationship with SMC differentiation. However, the physiological effect of Ang II was not related with inflammation.