Transcription factor TBX18 reprograms vascular smooth muscle cells of ascending aorta into pacemaker-like cells

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**Introduction**: Biological pacemaker is aimed to find a better replacement to treat bradycardia. Transcription factor TBX18 has been successfully applied for constructing biological pacemaker. And vascular smooth muscle cells (VSMCs) of the ascending aorta and SAN originated from the second heart field. The study explored whether ascending aortic smooth muscle cells in vitro could be reprogrammed into pacemaker-like cells with human TBX18.

**Methods**: The vascular smooth muscle cells (VSMCs) of ascending aorta were cultured by tissue block adherence. After 4-7 days, the cell morphology was observed under light microscope. After passaging, the cells were randomly divided into TBX18 group, GFP group and Null group. TBX18 group was transfected with adenovirus carrying TBX18 transcription factor and green fluorescent protein (GFP), and GFP group was transfected with equal amount of GFP adenovirus as empty virus. And blank group was not transfected with virus as control group (Null group). When transfected for 4 days, and then VSMCs were cocultured with neonatal rat ventricular cardiomyocytes (NRVMs) for 5 days in vitro. Three groups of transcription factors TBX3, human dwarf homeobox gene SHOX2, insulin gene enhancer binding protein 1 (Isl1), hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4), NKx2.5, Connexin 43(Cx43) and cardiomyocyte specificity cardiac troponin I (cTnI) firstly were detected by RT-qPCR and Western blot after 4 days. And the expression of HCN4 protein in TBX18 group and GFP group was detected by immunofluorescence. When VSMCs were cocultured for 5 days, funny current (If current) and action potentials were detected by the whole cell patch clamp and current clamp, respectively.

**Result**: The purity of vascular smooth muscle cells reached above 90% with α-SMA and MHC antibody. By overexpressing TBX18, the transfected VSMCs expressed high levels of TBX3, Shox2, Isl1, HCN4 and cTnI and low level of Cx 43 and NKx2.5 in both RT-qPCR and Western blot. The result of immunofluorescence showed that HCN4 protein (red fluorescence) in the TBX18 group was expressed and almost consistent with green fluorescent protein and cell nucleus (blue fluorescence), while the GFP group showed barely red fluorescence. In co-culture conditions, If current that recorded by patch clamp appeared the time and voltage dependence in TBX18 group, which the amplitude of If density was from -5.164±0.662 pA/pF to -0.765±0.358 pA/pF (n=14). Furthermore, the beating rate of TBX18-overexpressing VMScs was faster than in other groups in co-culture systems (178.00±7.55 bpm, P<0.05), and most importantly, the transfected cells by TBX18 exhibited sinoatrial-like APs which do not exist in the remaining groups.

**Conclusion**: Transcription factor TBX18 could reprogram vascular smooth muscle cells of ascending aorta into pacemaker-like cells in vitro.