Overexpression of the Intermediate-conductance Calcium-Activated Potassium Channel (SK4) and the HCN2 Channel to Generate a Biological Pacemaker

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**Introduction**: Ion channels play important roles in the excitation-contraction coupling of cardiac myocytes. Previous studies have shown that the overexpression or activation of intermediate-conductance calcium-activated potassium channel (SK4, encoded by KCNN4) in embryonic stem cell-derived cardiomyocytes (ESC-CM) can significantly increase their automaticity. The mechanism underlying this effect is probably related to the activation of hyperpolarized cyclic nucleotide gated channel (HCN) channels.

**Methods**: Ad-green fluorescent protein (GFP), Ad-KCNN4 and Ad-HCN2 recombinant adenoviruses were injected into the left ventricle of Sprague-Dawley(SD) rat hearts. The rats were divided into a GFP group (n=10), a SK4 group (n=10), a HCN2 group (n=10) and a SK4 plus HCN2 (SK4/HCN2) group (n=10). The isolated hearts were perfused after 5-7 days of gene expression, and a complete heart block model was established.

**Result**: Compared with the GFP group, overexpressing SK4 alone did not increase the heart rate after establishment of a complete heart block model (98.1±8.9 bpm, 96.7±7.6 bpm, P>0.05). The heart rates in the SK4/HCN2 (139.9±21.9 bpm) and HCN2 groups (111.7±5.5 bpm) were significantly increased compared with the GFP and SK4 groups, and the heart rates in the SK4/HCN2 group were significantly improved compared with the SK4 or HCN2 groups. In the HCN2 (n=8) and the SK4/HCN2 (n=7) groups, the shape of the spontaneous ventricular rhythm was the same as the ectopic rhythm after pacing the of the transgenically altered site. In contrast, these rhythms were different in the SK4 (n=10) and GFP (n=10) groups. There were no statistically significant differences in the APD alternans or ventricular arrhythmia inducibility between the four groups (all P>0.05). Western blotting, PCR and immunohistochemistry showed that the expression levels of SK4 and HCN2 increased significantly at the transgene site.

**Conclusion**: Biological pacemaker activity can be successfully generated by co-overexpression of SK4 and HCN2 without increasing the risk of ventricular arrhythmias. The overexpression of SK4 alone is insufficient to generate biological pacemaker activity. Our study provides evidence that SK4 and HCN2 combined could construct a ecotopic pacemaker, laying the groundwork for better construction biological pacing in the future.