Transcription factor prrx1 promotes brown adipose-derived stem cells differentiation to sinus node-like cells

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**Introduction**: Biological pacing is the production of a specific gene or cell-induced cell-like cell by genetic engineering to construct a pacing site similar to the atrial junction to replace the original damaged pacing cell, and to obtain the desired heart rate for the patient to be able to meet the normal physiological activity. Transfecting the adenovirus overexpressing prrx1 into BADSCs, our study aimed to investigate whether overexpression of prrx1 can successfully induce the differentiation of BADSCs into sinus node-like cells and construct biological pacing.

**Methods**: BADSCs of SD rats were isolated and cultured, and the cells were identified by flow cytometry when they were passaged to passages 3-5. The experimental groups were divided into two groups: BADSCs were transfected with empty adenovirus GFP and adenovirus prrx1 (ie, Ad-GFP group, Ad-prrx1 group). Cell morphology and fluorescence intensity were observed under fluorescence microscope. After 5-7 days of virus transfection, sinus node cell-associated pacing protein (HCN4) and ion channel (Cacnalg, encoding T-type calcium channel) as well as the expression levels of transcription factors (TBX18, ISL-1, pitx2, shox2, etc.) were detected by Western blot and RT-qPCR. Then, immunofluorescence assay to detect whether cell co-expressed prrx1 with HCN4, TBX18 and ISL-1. Finally, whole-cell patch clamp technique records pacing current If.

**Result**: The newly isolated cells were round, and after being attached to the wall, they were long fusiform and spirally growing. After identification by flow cytological cell surface molecules, the isolated cells showed CD90 positive and almost no CD45, indicating that BADSCs were successfully isolated from rats. Repeated experiments confirmed that the optimal MOI for adenovirus transfection of BADSCs was 100. After 5-7 days of transfection of adenovirus into cells, the biochemical tests showed that the mRNA levels and protein expressions of pacing-related factors (TBX18, ISL-1, HCN4, shox2, Cacnalg) in Ad-prrx1 group were significantly higher than those in Ad-GFP group. However, the expression level of pitx2 was decreased, and there was a statistical difference between the two groups (P<0.05). Immunofluorescence showed that prrx1 co-expressed with TBX18, ISL-1 and HCN4 in Ad-prrx1 group, but no expression of pacing-related protein was found in Ad-GFP group. Whole cell patch clamps were able to record the If current in the experimental group and this current was blocked by 4 mmol/L CsCl.

**Conclusion**: Overexpression of prrx1 can successfully induce the differentiation of BADSCs into sinus node-like cells with biochemical characteristics and electrophysiological characteristics.