An autofluorescence-based isolation of Leydig cells for testosterone deficiency treatment

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Statement of the Problem: Testosterone deficiency (TD) occurs when the serum testosterone levels are insufficient and can cause a series of clinical symptoms, including sexual dysfunction, obesity, muscle weakness and osteoporosis. Leydig cells (LCs) produce more than 95% of serum testosterone and the in-depth study of biological characteristics and regulatory mechanisms of LCs on testosterone production is helpful to elucidate the pathogenesis of TD. However, the density gradient centrifugation, as the currently main method for LC isolation, remain challenging. The purpose of this study is to identify the testicular autofluorescent cells and describe a simple and effective autofluorescence-based method for isolating LCs. Methodology: Testicular autofluorescent cells were isolated by FACS, and identified by qRT-PCR analysis and immunofluorescence staining. Then, the autofluorescent cells were further divided into two subpopulations by the combination of two fluorescence channels in FACS. The immunofluorescence staining, Live/Dead assay, cell counting, substrate utilization assay and LH stimulation assay were respectively used to evaluated the purity, viability, quantity and function of obtained LCs. Finally, the isolated LCs were subcutaneously implanted into castrated mice to evaluate the therapeutic potential of LCs *in vivo*. Findings: Testicular autofluorescent cells were composed of macrophages and LCs. Our autofluorescence-based method by the combination of two fluorescence channels successfully purified LCs from macrophages. Of note, the isolated LCs had high purity (>98%), viability (>98%) and quantity (approximately 4 × 105 cells per mouse) and maintained intact biochemical function. Moreover, subcutaneous transplantation of isolated LCs could relieve the symptoms of TD in castrated mice. Conclusion & Significance: we established a simple autofluorescence-based method that allows efficient isolation of well-functioning LCs with high purity, viability and quantity. This method can be used in detailed biological studies of LCs and will promote further advances in LC replacement therapies for TD.

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