

基础研究

脂质体介导人端粒酶逆转录酶基因构建永生化软骨细胞方法的建立及评价

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摘要:

目的: 探索将人端粒酶逆转录酶(hTERT)基因转入兔软骨细胞以获取大量种子细胞的方法和技术。方法: 分离培养原代兔软骨细胞,以pcDNA3.1/Zeo (+) -hTERT载体进行基因转染,对照组则为空载体pcDNA3.1/Zeo (+),筛选并挑选出阳性克隆后扩增,以逆转录-聚合酶链式反应(RT-PCR)检测转染hTERT基因后的软骨细胞hTERT mRNA表达;甲苯胺蓝染色判断转染hTERT基因后的软骨细胞是否具有软骨细胞的生物学特性;流式细胞术检测细胞周期及核型;裸鼠致瘤实验观察成瘤情况。结果:RT-PCR检测RNA水平上hTERT在细胞中的表达情况,hTERT转入兔软骨细胞后细胞形态未发生变化,长满单层后仍然呈现出多角形,与正常体外培养的软骨细胞形态基本一致。甲苯胺蓝染色后可见多糖染成蓝紫色,呈现较小蓝紫色颗粒,蛋白多糖等基质分布均匀,即转染hTERT基因后的软骨细胞具有合成和分泌软骨相关基质的功能。细胞周期测定转染hTERT的兔软骨细胞的增殖能力明显上升,细胞分裂基本以整数二倍体方式进行,保持了核型的基本稳定。裸鼠接种不致瘤。结论:转染hTERT的软骨细胞表型未发生转化,具有作为种子细胞和研究正常软骨细胞的生理、生化工具细胞的可行性。

关键词: 人端粒酶逆转录酶; 转染; 软骨细胞; 细胞增殖

Construction of immortalized chondrocytes by transfecting with human telomerase reverse transcriptase

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Abstract:

Abstract: Objective To explore the way and technique to gain adequate seed cells by introducing human telomerase reverse transcriptase(hTERT) gene into rabbit chondrocytes. Methods The chondrocytes of rabbit were isolated and cultivated, the expression vector pcDNA3.1/zeo (+) -hTERT was transfected into chondrocytes, in control group it was zero load pcDNA3.1/zeo (+), the positive clones were selected and amplified, the reverse transcription-polymerase chain reaction(RT-PCR) was used to detect the expression of hTERT mRNA, toluidine blue staining was used to analyze whether or not the chondrocytes transfected with hTERT gene possessed bionomics of chondrocytes. Flow cytometry examination was used to analyze cell cycle and karyotype. The condition of tumor formation of nude mouse was observed by tumorigenesis experiment. Results pcDNA3.1/zeo (+) -hTERT was transfected into chondrocytes detected by RT-PCR on the RNA level. The morphology of chondrocytes infected with pcDNA3.1/Zeo (+) -hTERT still kept polygon, which was same as the normal chondrocytes cultivated in vitro. After toluidine staining, polysaccharide was stained amethyst, small amethyst particles of proteoglycan were well-distributed. It suggested that the transformed cells still possessed the function of secreting cartilage correlated matrix. The multiplicate ability of transformed cells could be analyzed by measuring the cycle of cells. The multiplicate ability increased remarkably, the way of cell division was based on diploidy keeping the stabilization of karyotype. No tumorigenesis was found in nude mouse. Conclusion The proliferative ability of chondrocytes transfected with hTERT are enhanced obviously and the malignant transformation does not take place, they can serve as an excellent tool to study the biochemical and physiological aspect of chondrocytes and be used as seeding cells in tissue engineering.

Keywords: human telomerase reverse transcriptase; transfection; chondrocytes; cell proliferation

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