共培养体系中高红色荧光蛋白(RFP)标记结肠癌细胞的检测技术

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The technology of detecting high red fluorescence protein (RFP) labeled colon cancer cells in a co culture system

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摘要

目的 提高mCherry红色荧光蛋白(red fluorescence protein,RFP)在结肠癌细胞中的表达,从而可以通过检测共培养体系中细胞的荧光值来分析结肠癌细胞的增殖,以利于结肠癌肿瘤微环境的研究。方法 用293FT细胞制备pBMN-mCherry病毒液。利用多次感染(1~4次)的方法提高RFP在结肠癌细胞系HT29、LoVo、SW620及HCT116中的表达,经流式细胞仪检测得到不同感染次数细胞RFP标记的阳性率,并将高阳性表达RFP的结肠癌细胞HT29的荧光值与细胞数量关系进行比较,分析评价两者的线性关系。结果 通过优化,荧光标记效率大幅度提高。结肠癌肿瘤成纤维细胞共培养中,荧光值与细胞数量线性关系R2>0.9;该方法成功用于检测共培养体系中单种细胞增殖。结论 在结肠癌细胞和成纤维细胞的共培养体系中,结肠癌细胞的增殖可以通过检测细胞荧光值来分析。

关键词： 红色荧光蛋白(RFP), 共培养, 细胞增殖, 结肠癌

Abstract:

Objective To improve the expression efficiency of the mCherry red fluorescence protein (RFP) in colorectal cancer cells.So that the proliferation of colon cancer cells in the co-culture system can be easily monitored by measuring the fluorescence signals,which is suitable for colorectal cancer microenvironment analysis.Methods 293FT cells were used to produce the pBMN-mCherry virus,which was used to infect the colon cancer cell lines HT29,LoVo,SW620 and HCT116.The labeling procedure was repeated up to four times to improve the labeling efficiency of the RFP.The positively labeled cells of each round labeling were detected by flow cytometer.The fluorescence value of HT29 with highly labeled RFP was compared with its cell count and the linear relationship between both values was evaluated.Results After optimization,the fluorescence labeling efficiency was greatly improved.A linear coefficient correlation of R2>0.9 was achieved between the florescence value and the cell count when analyzing the proliferation of colon cancer cells in the co-cultured system.Conclusions In a co-culture system of colon cancer cells and fibroblasts,the proliferation of colon cancer cells can be measured by detecting their fluorescence signals.

Key words: red fluorescence protein (RFP) co-culture cell proliferation colorectal cancer

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