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论著

RFP标记的YCD基因在HeLa细胞中的表达及其介导的细胞毒性

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摘要 背景与目的：探讨红色荧光蛋白标记的酿酒酵母菌胞嘧啶脱氨酶(YCD)基因在人宫颈癌细胞(HeLa细胞)中的表达及其功能。材料与方法： 采用基因重组技术构建含有CMV启动子和RFP、YCD基因开放阅读框(ORF)的真核表达载体pIRES-YCD，脂质体法转染HeLa细胞。荧光显微镜下观察转染细胞中红色荧光蛋白的表达，流式细胞术分析转染效率及荧光强度并筛选荧光阳性细胞，命名为HeLa-Y。以不同浓度的前药5-FC处理HeLa-Y细胞，MTT法检测细胞存活率。 结果： 荧光显微镜下可见红色荧光蛋白在HeLa-Y细胞中表达，流式细胞术成功筛选出HeLa-Y细胞。前药5-FC能明显杀伤HeLa-Y细胞，5-FC浓度与HeLa-Y之间存在对数曲线关系。结论：RFP可作为报告基因快速筛选YCD表达载体转染的细胞，YCD/5-FC自杀基因系统可以进一步用于肿瘤的基因治疗研究。

关键词 红色荧光蛋白；酿酒酵母菌胞嘧啶脱氨酶；自杀基因；报告基因；基因表达.

Expression and Cytotoxicity of RFP-labeled Yeast Cytosine Deaminase in HeLa Cells

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Abstract BACKGROUND & AIM: To observe the expression of yeast cytosine deaminase (YCD) gene labeled by RFP in HeLa cells and how it mediates cytotoxicity. MATERIAL AND METHODS: YCD cDNA and RFP gene were cloned into Eukaryotic expression vector pIRES to construct YCD gene expression vector pIRES- YCD, then HeLa cells were transfected with pIRES-YCD in vitro. Expression of RFP gene in HeLa-Y cells was observed under a fluorescent microscope. The efficiency of transfection and the average intensity of fluorescence were analyzed by flow cytometry (FCM). The fluorescent cells were screened by FCM and were named HeLa-Y. The activity of HeLa-Y cells was detected by MTT colorimetric assay after they were treated with 5-FC at various concentrations.RESULTS: Expression of RFP gene in HeLa-Y cells was seen under the fluorescent microscope and HeLa-Y cells were screened by FCM. Apparently, the prodrug 5-FC killed HeLa-Y cells. There was logarithm cure relationship between 5-FC with HeLa-Y. CONCLUSION: These results demonstrate that RFP gene can be regarded as a reporter gene to screen the cells transduced with YCD quickly, and YCD/5-FC suicide gene system may prove helpful in gene therapy of cancer.

Keywords RFP YCD suicide gene reporter gene gene expression

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