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论文

人 PDCD4启动子真核报告质粒的构建和鉴定

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摘要：

目的 克隆人程序性死亡因子4(PDCD4)基因的启动子，插入荧光素酶报告基因载体中，并在细胞内检测其活性，为进一步研究PDCD4表达调控奠定基础。方法 采用PCR技术，从人基因组DNA中扩增出PDCD4启动子，插入荧光素酶报告基因载体pGL4-Basic中，测序所扩增的DNA序列，并将构建的pGL4-PDCD4-P1荧光素酶报告质粒，与内参照pRL-TK用脂质体法瞬时共转染OVCAR3，SKOV3细胞系，通过双荧光素酶活性检测确定其启动子活性。结果 测序结果表明，扩增的PDCD4启动子序列正确，pGL4-PDCD4-P1转染OVCAR3细胞(高表达内源性PDCD4) 24h后，双荧光素酶活性检测显示其启动子相对活性约为pGL4-Basic空载体的67倍；而pGL4-PDCD4-P1转染低表达内源性PDCD4的SKOV3细胞后相对活性约为15倍。结论 成功构建了PDCD4启动子的克隆及人PDCD4启动子报告基因，为后续研究奠定了基础。

关键词： 程度性死亡因子4；启动子；荧光素酶；报告基因

Construction and identification of the human PDCD4 promotor luciferase reporter plasmid

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Abstract:

Objective To construct the human programmed cell death 4 promotor luciferase reporter gene vector and detect its activity in cells. Methods The PDCD4 promotor from human genomic DNA was amplified by PCR, and was inserted into the luciferase report gene pGL4-basic vector. The amplified DNA sequence was confirmed by sequencing. To detect the transcriptional activity of human PDCD4-P1 in the plasmid,transient transfection was performed in different cell lines, and pRL-TK was used to determine the transfection efficiency. Results The sequencing results indicated that the amplified sequence was correct. The results of transient transfection showed that the recombinant plasmid could be highly expressed in the OVCAR3 cell line which could highly express endogenous PDCD4, but lowly in the SKOV3 cell line in which lowly expressed endogenous PDCD4 could be detected. Conclusion The human PDCD4 promotor luciferase reporter gene vector has been constructed successfully, which will lay experimental foundation for further study.

Keywords: Programmed cell death 4; Promotor; Luciferase; Reporter gene

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