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人PGI基因siRNA慢病毒质粒的构建及对白血病细胞

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Title: Construction of a lentiviral vector expressing human phosphoglucose isomerase gene siRNA and its influence on leukemia cell proliferation

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关键词: 白血病; 慢病毒载体; RNA干扰; 细胞增殖

Keywords: leukemia; lentiviral vector; siRNA; cell proliferation

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摘要: 目的 构建共表达绿色荧光蛋白（green fluorescent protein，GFP）基因和人磷酸葡萄糖异构酶（phosphoglucose isomerase，PGI）基因的慢病毒载体，初步探讨干扰人PGI基因对白血病细胞增殖的影响。 方法 RT-PCR、Western blot检测人单核细胞、K562、KG1-α、HL-60细胞中PGI mRNA和蛋白表达水平，筛选高表达PGI的白血病细胞系；构建人PGI基因siRNA的慢病毒载体及阴性对照，经293T细胞包装后，获得可表达人PGI基因siRNA的慢病毒载体及阴性对照；分别使用重组病毒（干扰组）、原始病毒（阴性对照组）和等量PBS（空白对照组）转染白血病KG1-α细胞，筛选稳定转染细胞株。RT-PCR和Western blot检测干扰组、阴性对照组及空白对照组KG1-α细胞PGI基因表达水平；CCK-8检测各组细胞增殖情况并作生长曲线。 结果 PGI基因在白血病KG1-a细胞中高表达；成功构建了稳定低表达PGI的白血病细胞株（KG1-α-siPGI）；低表达PGI基因的KG1-α细胞增殖能力较空白对照组明显降低，差异具有统计学意义（$P<0.05$）。 结论 成功构建了低表达PGI基因的白血病KG1-α细胞株，干扰PGI基因的表达能够抑制KG1-α细胞的生长。

Abstract: Objective To construct a lentiviral vector co-expressing green fluorescent protein (GFP) and human phosphoglucose isomerase (PGI), and to study the effect of human PGI gene interference on the proliferation of leukemia cells.

Methods RT-PCR and Western blotting were used to detect the mRNA and protein expression levels of PGI in human mononuclear cells, K562 cells, KG1-α cells and HL-60 cells, respectively, to screen a leukemia cell line with high expression of PGI. Recombinant human PGI gene siRNA lentiviral vector and its negative control were constructed and packaged by 293T cells, and the lentiviral vector that could express PGI siRNA in mammal cells were obtained. Recombinant lentivirus (interference group), original lentivirus (negative control group) and the same volume of phosphate buffer solution (PBS) (blank control group) were used to transfect KG1-α cells, respectively. After KG1-α cells with stable transfection were obtained, the mRNA and protein expression levels of PGI were detected by semi-quantitative RT-PCR and Western blotting, and cell proliferation was examined by CCK-8 assay. Results PGI was highly expressed in KG1-α cells, and KG1-α-siPGI cells with stable and low PGI expression were successfully constructed. Compared with the blank control group, the proliferation capability of KG1-α-siPGI cells decreased significantly (P<0.05). Conclusion We successfully construct the KG1-α leukemia cell line with low expression of PGI, and the interference of PGI expression can inhibit the proliferation of KG1-α cells.

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