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论著

siRNA靶向MIF重组逆转录病毒载体构建及稳定表达细胞株的筛选

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摘要 目的：构建并鉴定巨噬细胞移动抑制因子（MIF）特异性siRNA重组逆转录病毒载体，将其导入PHOENIX细胞中，筛选出分泌MIF-siRNA病毒的包装细胞，其分泌的病毒可以抑制MIF蛋白的表达，并初步了解稳定沉默MIF细胞株的特性。

方法:体外合成含针对MIF特异基因序列的寡核苷酸并定向克隆入pSuper.retro逆转录病毒载体，构建的载体通过酶切、测序鉴定后，采用脂质体介导转染包装病毒细胞株PHOENIX，收获病毒上清；将其感染HeLa细胞株，经过嘌呤霉素筛选得到抑制MIF表达的HeLa细胞株。Western blotting鉴定MIF表达的抑制效果并通过迁移实验、软琼脂糖克隆形成实验比较HeLa-pSuper-MIF和HeLa-pSuper-mock之间的特性。

结果:构建了重组逆转录病毒载体pSuper-MIF，转染包装病毒细胞株PHOENIX，病毒上清感染HeLa细胞，抑制了HeLa细胞内源性MIF蛋白的表达。沉默MIF蛋白后导致HeLa细胞迁移能力下降，不依赖支持物生长的特性减弱和黏附能力下降，同时稳定表达沉默MIF蛋白的细胞株生长周期停留在G 0/G 1期与对照组相比凋亡减少。

结论:构建了重组逆转录病毒载体pSuper-MIF，转染包装细胞所产生的病毒上清，能够抑制HeLa细胞内的MIF蛋白表达，表明MIF对HeLa细胞的迁移和不依赖支持物生长能力有重要作用。

关键词 巨噬细胞移动抑制因子； RNA干扰； 逆转录病毒载体

分类号 Q78

Construction of recombinant retroviral vector of short interfering RNAs specific for macrophage migration inhibitory factor (MIF) and establishment of stable HeLa cell line with a persistent knockdown of MIF

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Abstract

<FONT face=Verdana>AIM: To construct recombinant retroviral vector of short interfering RNAs (siRNA) specific for macrophage migration inhibitory factor (MIF) and to establish the stable knockdown of MIF cell line of mammalian cells by transfecting the recombinant retroviral vectors.
METHODS: We synthesized oligo-nucleotides for MIF in vitro, and cloned them into retroviral vector pSuper.retro. Subsequently the plasmids were sequenced and digested to identify the construction of the recombinant retroviral vectors. The vectors RNAi were transfected into packing cell line PHOENIX, which was selected by puromycin later. HeLa cell line was infected by the virus supernatant of stable PHOENIX cell lines, and the stable HeLa cell line showed significantly to silence MIF was established by selecting with puromycin. We also compare the characters of HeLa-pSuper-mock to HeLa-pSuper-MIF cells by using migration assay, adhesion assay, soft agar assay and FACS analysis of the cell-cycle progression.
RESULTS: The recombinant retroviral vectors were constructed successfully. The HeLa cell line infected by the supernatant containing the retrovirus of package PHOENIX cells was persistent knockdown of MIF confirmed by Western blotting. Knockdown of MIF in HeLa cells inhibited the migration and adhesion, and decreased the clone formation. FACS analysis revealed that knockdown of MIF arrested HeLa cells in G0/G1 phase.
CONCLUSION: We establish the stable HeLa cell line with a persistent knockdown of MIF. Our current studies reveal that MIF is necessary for HeLa cell

migration and anchorage-independent growth.</FONT>

Key words Macrophage migrotion-inhibitory factors RNA interference Retroviral vector

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