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慢病毒转染对鼻咽癌细胞 5-8F 增殖迁移的影响 *

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摘要 目的：探讨慢病毒转染对鼻咽癌细胞株 5-8F 增殖、迁移的影响，以验证慢病毒转染是否能有效的应用于鼻咽癌细胞的增值及迁移相关研究。**方法：**以红色荧光标记的慢病毒为转染载体，选定不同的 MOI 值转染鼻咽癌 5-8F 细胞株，扩大培养后筛选纯化，流式细胞仪检测转染效率。以最佳 MOI 值转染后的 5-8F（RFP-5-8F）细胞为实验组，未转染的亲代 5-8F 为空白对照组，取对数生长期未转染的亲代 5-8F 和红色荧光标记的慢病毒转染的 5-8F（RFP-5-8F）细胞进行 MTT、划痕实验，观察细胞镜下形态，了解细胞转染前后生长曲线，细胞迁移能力的变化。**结果：**流式细胞仪检测 5-8F 细胞慢病毒转染效率大于 95 %，转染最佳 MOI 值为 30，镜下荧光强度适中。实验组与对照组比较，转染前后 5-8F 细胞光镜形态相似，生长曲线一致，差异无统计学意义（P=0.997），划痕实验显示 5-8F 与 RFP-5-8F 细胞迁移能力一致，差异无统计学意义（P>0.05）。**结论：**慢病毒转染后鼻咽癌细胞能真实有效的的反应原细胞的增值及迁移能力，可以很好的应用于鼻咽癌增殖及其转移机制的相关研究。

关键词：鼻咽癌；5-8F 细胞；慢病毒；转染

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Effect of Lentivirus Transfection on the Proliferation and Migration of Nasopharyngeal Carcinoma Line 5-8F Cells*

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ABSTRACT Objective: To explore the effects of lentivirus transfection on the proliferation and migration of Nasopharyngeal carcinoma line 5-8F cells, and to verify whether lentivirus transfection can be effectively applied to the research for the proliferation and migration of Nasopharyngeal carcinoma cell line 5-8F cells. **Methods:** Different MOI values were selected to transfect Nasopharyngeal carcinoma line 5-8F cells with red fluorescence-labeled lentivirus as transfection vector. RFP-5-8F cells were cultured and then were purified after transfection. Transfection efficiency was determined by flow cytometry after transfection. RFP-5-8F cells transfected optimal MOI values was considered as the experimental group, and the untransfected parental 5-8F cells for the control group. MTT and Wound healing assays were used to detect the migration and proliferation of 5-8F and RFP-5-8F in logarithmic phase, and the morphology of these cells were observed under microscope. The cell growth curve and migration ability was analyzed. **Results:** Transfection efficiency of 5-8F cells were higher than 95 % by flow cytometry, and the best MOI was 30 with moderate endoscopic fluorescence intensity. Compared with that in the control group, 5-8F and RFP-5-8F cells in the experimental group were morphologically similar under microscope, and the growth curve of both were consistent, without statistical significance (P=0.997). Wound healing assays showed a consistent 5-8F and RFP-5-8F cell migration ability, without statistical significance (P>0.05). **Conclusion**: NPC cells after lentivirus transfection could effectively maintain their ability of proliferation and migration and could be applied to related mechanical research on the proliferation and metastasis of NPC.

Key words: Nasopharyngeal carcinoma; 5-8F cells; Lentivirus; Transfection

Chinese Library Classification(CLC): R739.6; R394.2 Document Code: A

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前言

肿瘤增值及侵袭转移相关基因的研究离不开基因载体的

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构建，常用基因导入的方法有物理法、化学法以及生物法[1]。因物理法和化学法转染效率较低，且对细胞有明显的损伤，目的基因不能稳定持续表达，目前研究少用。基因转染的载体包括磷酸钙、脂质体和多种阳离子物质介导、细菌质粒及病毒等，常用的病毒载体有腺病毒 (adenoviruses，Ad)、逆转录病毒(retroviruses，RV)、慢病毒(lentivimses，LV)等。腺病毒是一种双链DNA 病毒，其所携带的基因仅停留于细胞核中而不能整合到染色体上，因此，腺病毒介导的目的基因不能持久表达，仅能维持数天到数周。逆转录病毒载体仅能感染分裂期细胞，而且容量有限。慢病毒是Ⅰ型人类免疫缺损病毒(HIV-1)，其与逆转录病毒均为 RNA 病毒，与一般的逆转录病毒载体不同，其免疫反应较小，目的基因持久表达，能够转染非分裂期细胞。有研究表明，慢病毒对终末分化的神经细胞均有感染能力[2]，在大鼠脑内经慢病毒载体介导的蛋白表达可长达六个月，而且没有任何毒性[3,4]。因此，近年来慢病毒载体越来越受到研究者的青睐，被广泛应用于包括鼻咽癌在内的肿瘤相关研究中[5,6]。但亦有研究者认为，病毒载体在携带基因转染本身可能对细胞的形态、生物学特性、功能有影响。张迎春等研究表明，人巨细胞病毒可造成体外培养的脐血粒 - 单祖细胞集落染色体的损伤 [7]；高岚等研究巨细胞病毒感染孕妇治疗前后外周血染色体着丝粒点(Cd)变异率的变化，结果显示，巨细胞病毒是导致外周血着丝粒点畸变的因素[8]。慢病毒载体随机插入细胞基因组中[9]，所以排除转染过程对细胞其他生物学特性和表型的影响是必要的[10]。目前，尚没有关于慢病毒载体对鼻咽癌细胞株是否有影响的报道，因此，本实验拟通过红色荧光标记的慢病毒载体转染具有转移潜能的鼻咽癌细胞株 5-8F，以探索慢病毒载体对肿瘤细胞增殖、迁移的影响，从而验证慢病毒转染是否能有效的应用于鼻咽癌细胞的增值及迁移相关研究。

1 材料与方法

1.1 红色荧光标记的慢病毒转染

取 5-8F 细胞，加入含 10 %小牛血清的 1640 培养基，在 37 ℃，体积分数为 5 %的 CO_2 饱和湿度恒温箱中培养，生长至 80 %~90 %融合度时，用胰酶消化，计数，96 孔板接种 3.5× 10^3/孔。过夜贴壁后，加入慢病毒转染体系 100 μL，MOI 值分别为 1、5、10、15、30、40、50、60、70、80，每个 MOI 值做 3 个复孔，培养 10 小时后吸出病毒转染体系(参考 Lentiviral Vector Particle 使用操作手册)，加入培养基，48 小时后显微镜下观察，扩大培养。以转染红色荧光的 5-8F 细胞为实验组，未转染的亲代 5-8F 细胞为空白对照组，实验组与对照组同条件培养，同时期观察。以空白对照组 5-8F 细胞为质控标准，行流式细胞检测仪测定实验组 RFP-5-8F(Red Fluorescent Protein)细胞转染效率，检测后进一步纯化扩大培养。

1.2 MTT 细胞增殖实验

分别收集处于对数生长期的 5-8F，RFP-5-8F 细胞，96 孔板接种 1000 个 / 孔，共分两组，每组细胞种 36 个复孔，边缘孔用无菌 PBS 填充，37 ℃、5 % CO_2 条件下培养，每隔 24 小时选取 12 孔进行 MTT 检测，具体为加入新鲜配制 5 g / L MTT 溶液 20 μL，再培养 4 小时后吸尽废液，每孔加入 150 μL DMSO，避光振荡 10 分钟，结晶甲瓒充分溶解后，经酶标仪 A570 处测定各孔吸光度值。共测定 6 天。实验重复 3 次。

1.3 转染前后 5-8F，RFP-5-8F 细胞划痕实验

6 孔板背后用标记笔靠直尺均匀画线横穿过孔，每孔 5 条线，间隔约 0.5～1 cm。取对数生长期 5-8F，RFP-5-8F，调整细胞悬液浓度每孔种细胞 5× 10^5 个，过夜铺满 6 孔板。20 μL 进口枪头高压灭菌，垂直于六孔板背后所画横线均匀用力、匀速划痕，加 PBS 轻晃洗去划下的细胞，加入 1 %胎牛血清的 1640 培养，按 0、6、12、24 小时取样显微镜下拍照及红色荧光照片，采用 Image pro plus 软件测量各组细胞迁移距离。6 小时划痕愈合率 =（0 小时划痕距离 -6 小时划痕距离）/ 0 小时划痕距离，12、24 小时愈合率同理。

1.4 统计学处理

数据结果以均数± 标准差（$\bar{x}$± s）表示，使用统计学软件 SPSS 16.0 处理，两独立样本均数的比较用 t 检验分析，P<0.05 为差异显著，有统计学意义。

2 结果

2.1 转染前后细胞的镜下形态

红色荧光蛋白标记的慢病毒转染前后，空白对照组 5-8F 细胞，实验组 RFP-5-8F 细胞光镜下细胞形态结构无明显差异，如图 1。

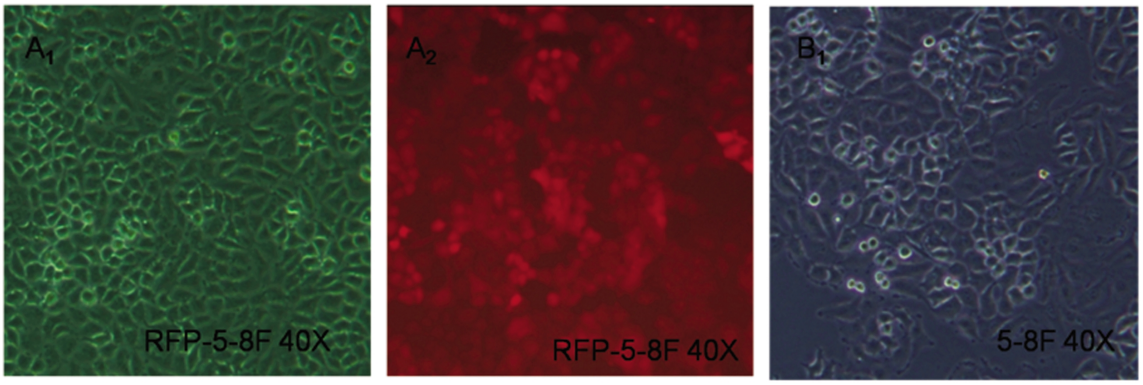

图 1 转染前后 5-8F 显微镜下照片

Fig. 1 Micrograph of 5-8F before and after transfection

注：A1 为红色荧光蛋白标记的慢病毒转染后 RFP-5-8F 细胞明场观察；A2 为红色荧光蛋白标记的慢病毒转染后 RFP-5-8F 细胞荧光观察；B1 为未转染的亲代 5-8F 细胞明场观察。

Note: A1: Light micrograph of Red Fluorescent Protein-5-8F after transfection; A2: Fluorescence micrographs of Red Fluorescent Protein-5-8F after transfection; B1: Light micrograph of 5-8F before transfection.

2.2 5-8F 转染结果及转染效率的测定

转染 48 小时后显微镜观察开始出现荧光，72 小时左右荧光强度达到稳定且趋强度最强。镜下观察 MOI 值为 30 转染的细胞荧光强度可且细胞转染后细胞死亡数量较少，选 MOI 值为 30 行转染效率测定。流式细胞检查仪检查转染效率大于 95%，如图 2。

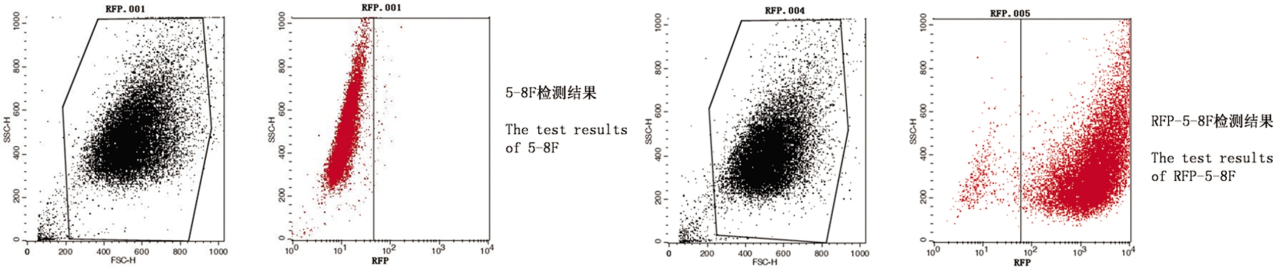

图 2 转染后流式细胞仪转染效率的测定

Fig. 2 Transfection efficiency by flow cytometry after transfection

2.3 转染前后细胞增殖情况

MTT 结果显示，5-8F，RFP-5-8F 细胞生长曲线基本相似，如图 3，差异无统计学意义，t=-0.004，P=0.997。

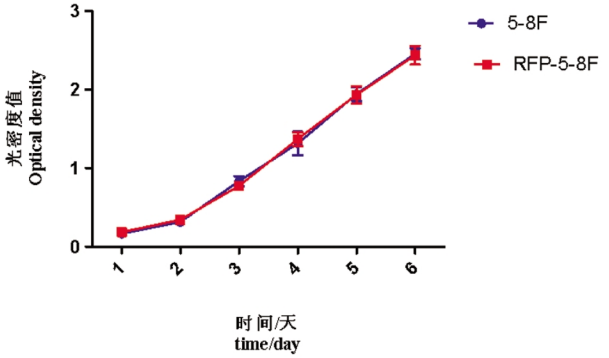

图 3 转染前后 5-8F 生长曲线

Fig. 3 Growth curve of 5-8F before and after transfection

2.4 转染前后细胞划痕实验

划痕实验显示，5-8F，RFP-5-8F 细胞划痕愈合速度相近，划痕愈合率相近，差异无统计学意义（P>0.05），如图 4、表 1、图 5。

3 讨论

慢病毒载体是一类重组逆转录病毒载体，由于其结构和功能的特点，作为一种重要的基因转移工具被应用于基因治疗等细胞分子生物学研究领域[11,12]。慢病毒载体较逆转录病毒载体

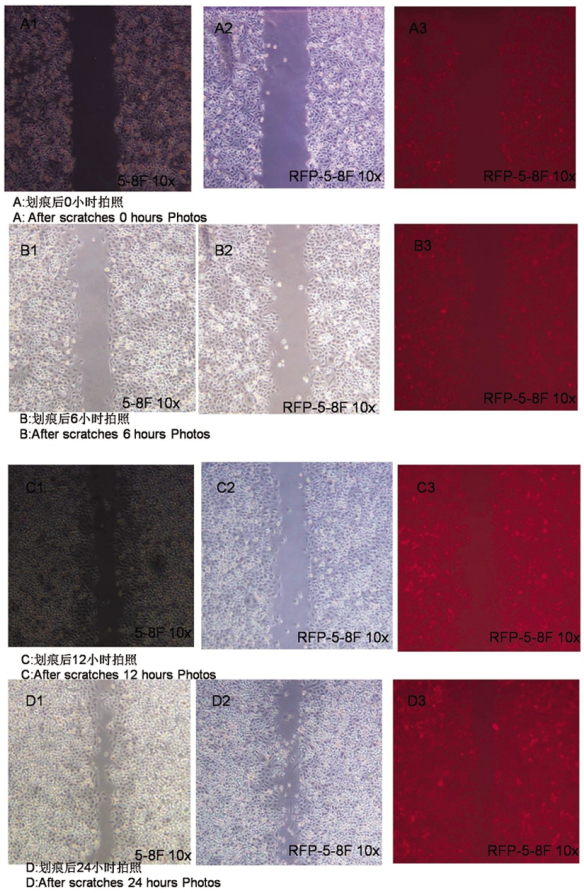

图 4 划痕实验照片

Fig. 4 Photos for wound healing assay

表 1 慢病毒转染对 5-8F 细胞迁移能力的影响

Table 1 Effects of lentivirus transfection on cell migration ability of 5-8F

Time(after scratches)	The rate of wound healing(%)		P values
	5-8F	RFP-5-8F	
6 hours	26.67± 1.00	25.54± 1.93	0.419
12 hours	41.26± 1.41	40.39± 0.68	0.394
24 hours	72.21± 3.63	72.29± 2.26	0.977

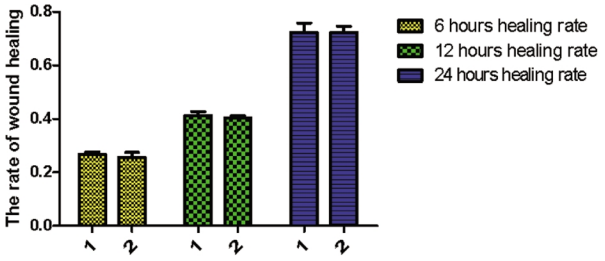

图 5 5-8F 转染前后划痕实验划痕愈合率直方图

Fig. 5 Wound healing rate histograms of 5-8F before and after transfection

注:1 为 5-8F 细胞,2 为 RFP-5-8F 细胞。

Note:1: 5-8F cells; 2: RFP-5-8F cells.

有更广的宿主范围,能够将目标基因片段转染到受精卵、干细胞,及已分化的细胞中[13,14],稳定转染不同种类的细胞,让特定基因在实验小鼠体内持久稳定的表达 [15], 为在细胞组织中快速、高效的研究特定基因的表达提供了有效的工具。慢病毒载体可用于研究特定细胞株蛋白质的表达、微小 RNA(miRNA)与肿瘤的联系及 RNA 干扰等[16-18]。

携带插入宿主基因组的特定目的基因的慢病毒载体对于转录沉默作用具有抵抗能力,对于活体动物转染实验、培养细胞转染实验,由慢病毒载体携带导入的目的基因均能在宿主细胞中持久而稳定的表达[19],这为后续实验提供了明确的观察指标,有利于后续研究的顺利进行。鼻咽癌的浸润、转移癌细胞易脱落、溶解和侵入周围组织,或侵入血管、淋巴管,随血流或淋巴液播散到远处,并在其他部位形成新的癌瘤。携带红色荧光蛋白的 5-8F 细胞有利于肿瘤细胞的追踪研究。

慢病毒转染细胞的效率受细胞膜表面脂质体的含量、启动子在细胞内的表达强度的影响,因此不同的种类的细胞有不同的最佳 MOI 值(感染病毒与细胞数量的比值)。MOI 是影响细胞的转染效率的因素, 过高的 MOI 值可导致细胞的死亡及裂解[20],通过镜下观察荧光强度及流式细胞检测转染效率,发现最佳慢病毒转染 5-8F 的最佳 MOI 值为 30。本实验采用 MTT 法测定 5-8F,RFP-5-8F 细胞生长曲线图 3 所示,统计发现转染前后细胞增殖能力无明显差异,细胞划痕实验提示细胞转染前后的迁移能力无明显变化(如图 4 所示),提示转染 RFP 标记的慢病毒未使 5-8F 细胞的增值、迁移等生物学行为发生改变。因此, 慢病毒转染红色荧光蛋白对 5-8F 细胞的生物学特性无显著影响,可用于后继实验,为鼻咽癌的转移、淋巴转移研究及动物模型的构建提供了有效的工具。

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