

## 单核细胞趋化蛋白1对肺癌A549细胞迁移和侵袭的影响及其机制

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**[摘要]** **目的:** 探讨单核细胞趋化蛋白1 (MCP-1) 对肺癌A549细胞迁移和侵袭的影响, 并阐明其作用机制。**方法:** 采用免疫组织化学法检测80例非小细胞肺癌 (NSCLC) 及癌旁正常肺组织中MCP-1蛋白表达情况。体外培养人肺癌A549细胞, MCP-1-小干扰RNA (siRNA) 实验分为空白组、阴性对照组 (si-NC组)、MCP-1-siRNA-1组和MCP-1-siRNA-2组; MCP-1过表达实验分为对照组、空载对照组 (OE-NC组, 转染MCP-1过表达空载质粒)、过表达MCP-1组 (OE-MCP-1组, 转染MCP-1过表达质粒)、过表达MCP-1+细胞外调节蛋白激酶 (ERK) 信号通路抑制剂PD98059组 (OE-MCP-1+PD98059组, 共转染MCP-1过表达质粒和PD98059) 和PD98059组 (转染PD98059)。分别将MCP-1-siRNA和质粒转染至肺癌A549细胞, Western blotting法验证各组A549细胞转染效率。细胞划痕实验和Transwell小室实验观察各组A549细胞迁移率和侵袭细胞数, Western blotting法检测各组A549细胞中磷酸化ERK (p-ERK)、总ERK (t-ERK) 和上皮-间质转化 (EMT) 相关蛋白表达水平。**结果:** 与癌旁组织比较, NSCLC组织中MCP-1蛋白阳性表达率明显升高 ( $P<0.05$ ); NSCLC组织中MCP-1蛋白表达水平与TNM分期和淋巴结转移有关联 ( $P<0.05$ )。与si-NC组比较, MCP-1-siRNA-1组和MCP-1-siRNA-2组A549细胞中MCP-1蛋白表达水平均明显降低 ( $P<0.01$ ); 与对照组和OE-NC组比较, OE-MCP-1组A549细胞中MCP-1蛋白表达水平明显升高 ( $P<0.01$ )。细胞划痕实验, 与si-NC组比较, MCP-1-siRNA-1组和MCP-1-siRNA-2组细胞迁移率均明显降低 ( $P<0.01$ ); 与OE-NC组比较, OE-MCP-1组细胞迁移率明显升高 ( $P<0.01$ ); 与OE-MCP-1组比较, OE-MCP-1+PD98059组细胞迁移率明显降低 ( $P<0.01$ ); 与OE-MCP-1+PD98059组比较, PD98059组细胞迁移率明显降低 ( $P<0.01$ )。Transwell小室实验, 与si-NC组比较, MCP-1-siRNA-1组和MCP-1-siRNA-2组侵袭细胞数明显减少 ( $P<0.01$ ); 与OE-NC组比较, OE-MCP-1组侵袭细胞数明显增加 ( $P<0.01$ ); 与OE-MCP-1组比较, OE-MCP-1+PD98059组侵袭细胞数明显减少 ( $P<0.01$ ); 与OE-MCP-1+PD98059组比较, PD98059组侵袭细胞数明显减少 ( $P<0.01$ )。Western blotting法, 与si-NC组比较, MCP-1-siRNA-1组和MCP-1-siRNA-2组A549细胞中p-ERK、波形蛋白 (Vimentin) 和N-钙黏蛋白 (N-cadherin) 表达水平均明显降低 ( $P<0.05$ 或 $P<0.01$ ), E-钙黏蛋白 (E-cadherin) 表达水平均明显升高 ( $P<0.01$ ); 与OE-NC组比较, OE-MCP-1组A549细胞中p-ERK、Vimentin和N-cadherin蛋白表达水平均明显升高 ( $P<0.01$ ), E-cadherin蛋白表达水平均明显降低 ( $P<0.01$ ); 与OE-MCP-1组比较, OE-MCP-1+PD98059组A549细胞中p-ERK、Vimentin和N-cadherin蛋白表达水平均明显降低 ( $P<0.01$ ), E-cadherin蛋白表达水平均明显升高 ( $P<0.05$ ); 与OE-MCP-1+PD98059组比较, PD98059组A549细胞中p-ERK、Vimentin和N-cadherin蛋白表达水平均明显降低 ( $P<0.05$ 或 $P<0.01$ ), E-cadherin蛋白表达水平明显升高 ( $P<0.01$ )。**结论:** MCP-1蛋白可上调肺癌A549细胞中EMT相关蛋白表达, 促进肺癌A549细胞的迁移和侵袭, 其作用机制与激活ERK信号通路有关。

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## Effects of monocyte chemoattractant protein-1 on invasion and migration of lung cancer A549 and their mechanisms

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**ABSTRACT Objective:** To discuss the effects of monocyte chemoattractant protein-1 (MCP-1) on the migration and invasion of lung cancer A549 cells, and to clarify the mechanisms. **Methods:** Immunohistochemistry method was used to detect the expression of MCP-1 protein in 80 cases of non-small cell lung cancer (NSCLC) and adjacent normal lung tissues. The human lung cancer A549 cells were cultured *in vitro*. The MCP-1-small interfering RNA (siRNA) experiment was divided into blank group, negative control group (si-NC group), MCP-1-siRNA-1 group, and MCP-1-siRNA-2 group. The MCP-1 over-expression experiment was divided into control group, empty vector control group (OE-NC, transfected with MCP-1 over-expression empty vector), over-expression MCP-1 group (OE-MCP-1 group, transfected with MCP-1 over-expression plasmid), over-expression MCP-1+extracellular regulated protein kinase (ERK) pathway inhibitor PD98059 group (OE-MCP-1+PD98059 group, co-transfected with MCP-1 over-expression plasmid and PD98059), and PD98059 group (transfected with PD98059). The MCP-1 siRNA and plasmids were transfected into the lung cancer A549 cells; Western blotting method was used to verify the transfection efficiencies of the cells in various groups; the migration rate and the number of invasion cells in various groups were observed by wound healing assay and Transwell chamber assay, respectively; Western blotting method was also used to detect the expression levels of phosphorylated ERK (p-ERK), total ERK (t-ERK), and epithelial-mesenchymal transition (EMT)-related proteins in the A549 cells in various groups. **Results:** Compared with adjacent tissue, the positive expression rate of MCP-1 protein in NSCLC tissue was significantly increased ( $P<0.05$ ), and the expression level of MCP-1 protein was related to TNM stage and lymph node metastasis ( $P<0.05$ ). Compared with si-NC group, the expression level of MCP-1 protein in the cells in MCP-1-siRNA-1 and MCP-1-siRNA-2 groups was significantly decreased ( $P<0.01$ ). Compared with control group and OE-NC group, the expression level of MCP-1 protein in the cells in OE-MCP-1 group was significantly increased ( $P<0.01$ ). The wound healing assay results showed that compared with si-NC group, the migration rate of the cells in MCP-1-siRNA-1 and MCP-1-siRNA-2 groups were significantly decreased ( $P<0.01$ ). Compared with OE-NC group, the migration rate of the cells in OE-MCP-1 group was significantly increased ( $P<0.01$ ); compared with OE-MCP-1 group, the migration rate of the cells in OE-MCP-1+PD98059 group was significantly decreased ( $P<0.01$ ). Compared with OE-MCP-1+PD98059 group, the migration rate of the cells in PD98059 group was significantly decreased ( $P<0.01$ ). The Transwell chamber assay results showed that compared with si-NC group, the number of invasion cells in MCP-1-siRNA-1 and MCP-1-siRNA-2 groups was significantly decreased ( $P<0.01$ ). Compared with OE-NC group, the number of invasion cells in OE-MCP-1 group was significantly increased ( $P<0.01$ ); compared with OE-MCP-1 group, the number of invasion cells in OE-MCP-1+PD98059 group was

significantly decreased ( $P < 0.01$ ); compared with OE-MCP-1+PD98059 group, the number of invasion cells in PD98059 group was significantly decreased ( $P < 0.01$ ). The Western blotting results showed that compared with si-NC group, the expression levels of p-ERK, Vimentin, and N-cadherin protein in the cells in MCP-1-siRNA-1 and MCP-1-siRNA-2 groups were significantly decreased ( $P < 0.05$  or  $P < 0.01$ ), and the expression level of E-cadherin proteins was significantly increased ( $P < 0.01$ ). Compared with OE-NC group, the expression levels of p-ERK, Vimentin, and N-cadherin proteins in the cells in OE-MCP-1 group were significantly increased ( $P < 0.01$ ), and the expression level of E-cadherin protein was significantly decreased ( $P < 0.01$ ). Compared with OE-MCP-1 group, the expression levels of p-ERK, Vimentin, and N-cadherins proteins in the OE-MCP-1+PD98059 group were significantly decreased ( $P < 0.01$ ), and the expression level of E-cadherin protein was significantly increased ( $P < 0.05$ ). Compared with OE-MCP-1+PD98059 group, the expression levels of p-ERK, Vimentin, and N-cadherin proteins in the cells in PD98059 group were significantly decreased ( $P < 0.05$  or  $P < 0.01$ ), and the expression level of E-cadherin protein was increased ( $P < 0.01$ ). **Conclusion:** MCP-1 protein can upregulate the expression of EMT-related proteins in the lung cancer A549 cells, and promote the migration and invasion of the lung cancer A549 cells; its mechanism may be related to the activation of the ERK signaling pathway.

**KEYWORDS** Monocyte chemoattractant protein-1; Extracellular-signal regulated protein kinase; Cancer, non-small cell lung; Cell invasion; Cell migration

肺癌是世界上最常见的恶性肿瘤之一, 其中80%~85%为非小细胞肺癌(non-small cell lung cancer, NSCLC)。因其易复发、微转移和预后差等特点, 肺癌患者5年生存率较低<sup>[1]</sup>。深入研究肺癌侵袭与转移的发生机制可为肺癌治疗提供新的作用靶点。单核细胞趋化蛋白1(monocyte chemoattractant protein-1, MCP-1)属于趋化因子家族, 参与调控机体炎症。研究<sup>[2-5]</sup>显示: MCP-1在多种肿瘤组织中过表达, 可能通过激活CC趋化因子受体(CC chemokine receptor, CCR)2或CCR4, 经细胞外调节蛋白激酶(extracellular regulated protein kinase, ERK)、蛋白激酶B(protein kinase B, AKT)和信号转导和转录激活因子3(signal transducer and activator of transcription 3, STAT3)等信号通路诱导上皮-间质转化(epithelial-mesenchymal transition, EMT), 参与肿瘤细胞的侵袭和转移。目前, 关于MCP-1调控肺癌细胞侵袭和转移的作用机制尚未完全阐明。本研究探讨MCP-1对NSCLC A549细胞迁移和侵袭的影响, 初步阐明MCP-1调控肺癌细胞发生EMT的信号通路, 为肺癌治疗提供新的思路。

## 1 资料与方法

**1.1 一般资料** 收集80例2020年1—12月来源于锦州医科大学附属第一医院病理科的NSCLC标本。本研究已获得锦州医科大学伦理委员会批准。选取经病理学证实的NSCLC标本和距肿瘤边缘

5 cm的癌旁正常肺组织。所有选取患者在术前均未接受过化疗及放疗。

**1.2 细胞、主要试剂和仪器** 人肺癌细胞株A549购于中国科学院上海细胞库。RPMI-1640培养基购自美国Gibco公司, 胎牛血清购自美国CLARK Bioscience公司, MCP-1-小干扰RNA(small interfering RNA, siRNA)购自苏州吉玛生物有限公司, MCP-1过表达质粒GV657-MCP-1购自上海吉凯基因公司, Lipofectamine 3000购自美国Invitrogen公司, MCP-1、波形蛋白(Vimentin)、N-钙黏蛋白(N-cadherin)、E-钙黏蛋白(E-cadherin)和 $\beta$ -actin等抗体均购自美国SAB公司, 磷酸化ERK(phosphorylated ERK, p-ERK)和总ERK(total ERK, t-ERK)抗体购自沈阳万类生物技术有限公司, ERK信号通路抑制剂PD98059购自美国MCE公司, Matrigel基底胶购自美国BD公司, Transwell小室购自美国康宁生物技术有限公司, BCA蛋白浓度测定试剂盒购自上海碧云天公司, 免疫组织化学染色用MCP-1抗体购自美国Affinity公司, 即用型免疫组织化学超敏SP试剂盒、柠檬酸缓冲液和浓缩DAB试剂盒购自福建迈新生物技术有限公司。细胞CO<sub>2</sub>培养箱(型号: Thermo Heracell 1501)购自美国Thermo公司, 垂直电泳仪和转膜仪(型号: Bio-rad 1645050)购自美国伯乐公司, 显影仪(型号: Amersham Imager 600)购自日本GE公司, 数字切片扫描与

应用系统(型号: Easy scan NFC)购自厦门麦克奥迪实业集团有限公司。

**1.3 免疫组织化学法检测 NSCLC 组织和癌旁正常肺组织中 MCP-1 蛋白表达情况** 石蜡切片常规脱蜡至水, 并于 pH 值为 6.0 的柠檬酸修复液中经高压抗原修复 3.5 min, 3% H<sub>2</sub>O<sub>2</sub> 室温下孵育 10 min, 清除内源性过氧化物酶活性, 磷酸缓冲盐溶液(phosphate buffer saline, PBS)冲洗 3 次, MCP-1 一抗 4 °C 冰箱过夜。次日 PBS 缓冲液冲洗 3 次, 滴加二抗工作液。新鲜配制的 DAB 显色, 苏木素复染 7 min, 自来水反蓝 30 min, 脱水透明后树脂封片。免疫组织化学染色结果判定标准: MCP-1 阳性表达主要定位于细胞质, 以其内出现棕黄色细颗粒状为阳性。由 2 位医师采用双盲法观察每张切片, 对每张切片随机选取 5 个高倍视野。将切片中阳性细胞数占细胞总数的百分率计为分数 1: 0 分, 无阳性细胞; 1 分, 阳性细胞百分率 < 25%; 2 分, 阳性细胞百分率 ≥ 25% 但 < 50%; 3 分, 阳性细胞百分率 ≥ 50%。按细胞中细胞显色强度计为分数 2: 0 分, 细胞无显色; 1 分, 细胞呈浅黄色(弱染色); 2 分, 细胞呈棕黄色(中等染色); 3 分, 细胞呈棕褐色(强染色)。分数 1 × 分数 2 ≥ 3 分计为阳性, < 3 分计为阴性。MCP-1 蛋白阳性表达率 = MCP-1 蛋白阳性细胞数 / 总细胞数 × 100%。

**1.4 细胞培养、分组和转染** 肺癌 A549 细胞培养于含 10% 胎牛血清和 1% 青-链霉素的 RPMI-1640 培养基中, 37 °C 恒温、5% CO<sub>2</sub> 细胞培养箱中常规培养传代。MCP-1-siRNA 实验分为空白组、阴性对照组 (si-NC 组)、MCP-1-siRNA-1 组和 MCP-1-siRNA-2 组。细胞培养至密度为 40%~50% 时, 使用 Lipofectamine 3000 包裹 MCP-1-siRNA 干扰序列。siRNA 序列: MCP-1-siRNA-1 组, 上游引物, 5'-GCUCAUAGCAGCCACCUUCTT-3', 下游引物, 5'-GAAGGUGGCUGCUAUGAGCTT-3'; MCP-1-siRNA-2 组, 上游引物, 5'-CAAACCCA-AACUCCGAAGATT-3', 下游引物, 5'-UCUUC-GGAGUUUGGGUUUGTT-3'; 阴性对照组, 上游引物, 5'-UUCUCCGAACGUGUCACGUTT-3', 下游引物, 5'-ACGUGACACGUUCGGAGAATT-3'。构建 MCP-1 过表达质粒 GV657-MCP-1, 细胞培养至密度为 70%~90% 时, 使用 Lipofectamine 3000 转染。过表达 MCP-1 实验分为对照组、空载对照组 (OE-NC 组, 转染 MCP-1 过表达空载质粒)、

过表达 MCP-1 组 (OE-MCP-1 组, 转染 MCP-1 过表达质粒)、过表达 MCP-1+PD98059 组 (OE-MCP-1+PD98059 组, 共转染 MCP-1 过表达质粒和 PD98059) 和 PD98059 组 (转染 PD98059)。PD98059 浓度为 10 μmol·L<sup>-1</sup>。转染 4~6 h 后更换为完全培养基继续培养, 采用 Western blotting 法验证各组 A549 细胞转染效率。

**1.5 细胞划痕实验检测各组肺癌 A549 细胞迁移率** 收集处于对数期细胞, 按每孔 4 × 10<sup>5</sup> 个细胞的密度接种于 6 孔细胞培养板。转染 24 h 后, 待细胞生长至密度约 90% 时, 使用 200 μL 枪头划痕, PBS 缓冲液洗涤 3 次。0 h 时显微镜下观察并拍照记录, 继续培养 24 h 后拍照, 观察细胞迁移情况, 实验重复 3 次, 计算各组 A549 细胞迁移率。细胞迁移率 = (0 h 划痕面积 - 24 h 划痕面积) / 0 h 划痕面积 × 100%。

**1.6 Transwell 小室实验检测各组肺癌 A549 细胞侵袭细胞数** Matrigel 胶和 RPMI-1640 培养液按 1: 9 混合, 上室每孔加入 80 μL 已稀释的 Matrigel 胶, 置于 37 °C、5% CO<sub>2</sub> 培养箱培养 30 min, 待其聚合成胶。转染 24 h 后无血清培养基重悬细胞, 调整细胞密度为 5 × 10<sup>5</sup> mL<sup>-1</sup>。上室每孔加入 200 μL 细胞悬液, 下室加入 600 μL 完全培养基。24 h 后吸去上室培养基, PBS 缓冲液洗涤 2 次, 75% 甲醇固定 30 min, PBS 缓冲液洗涤 2 次, 0.1% 结晶紫染色 20 min, PBS 缓冲液洗涤 2 次。棉签擦去上室未透膜的细胞, 晾干小室。倒置显微镜下观察, 随机选取 5 个视野拍照并计数, 计算各组 A549 细胞侵袭细胞数。

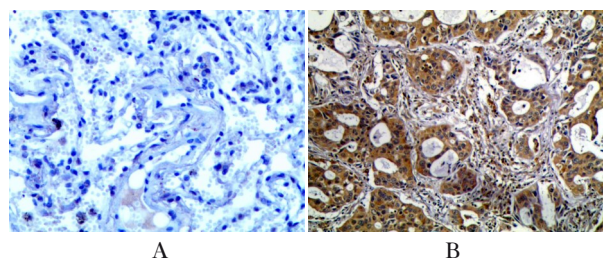
**1.7 Western blotting 法检测各组肺癌 A549 细胞中 p-ERK、t-ERK 和 EMT 相关蛋白表达水平** 转染 48 h 后收集并裂解细胞, 离心收集上清液, BCA 试剂盒测定蛋白浓度。经 SDS-聚丙烯酰胺凝胶电泳 (polyacrylamide gel electrophoresis, PAGE), 聚偏二氯乙烯 (polyvinylidene difluoride, PVDF) 转膜, 5% 脱脂牛奶室温封闭 2 h 后, 分别加入相应的一抗, 4 °C 孵育过夜。次日, TBST 溶液洗去一抗, 室温孵育二抗 1 h, TBST 溶液洗涤后加入 ECL 发光试剂, 显影, 采用 Image J 软件分析蛋白条带灰度值, 以 β-actin 为内参, 计算目的蛋白表达水平。目的蛋白表达水平 = 目的蛋白条带灰度值 / 内参蛋白条带灰度值。

**1.8 统计学分析** 采用 Graphpad Prism 9.0.2 统

计软件进行统计学分析。不同临床病理特征(包括性别、年龄、肿物最大径、组织学类型、分化程度、TNM分期和淋巴结转移情况等)患者NSCLC组织中MCP-1蛋白表达情况以例数表示,采用 $\chi^2$ 检验。各组肺癌A549细胞迁移率、侵袭细胞数和MCP-1、ERK及EMT相关蛋白表达水平均符合正态分布,以 $\bar{x}\pm s$ 表示,多组间样本均数比较采用单因素方差分析,组间样本均数两两比较采用SNK-*q*检验。以 $P<0.05$ 为差异有统计学意义。

## 2 结果

**2.1 NSCLC组织和癌旁正常肺组织中MCP-1蛋白阳性表达率** 与癌旁正常肺组织(26.2%)比较,NSCLC组织中MCP-1蛋白阳性表达率(62.5%)明显升高( $P<0.05$ )。NSCLC组织中MCP-1蛋白表达水平与TNM分期及淋巴结转移有关联( $P<0.05$ )。见图1和表1。



A: Adjacent normal lung tissue; B: NSCLC tissue.

图1 NSCLC组织和癌旁正常肺组织中MCP-1蛋白表达情况(免疫组织化学,×200)

Fig. 1 Expressions of MCP-1 protein in NSCLC tissue and adjacent normal lung tissue(Immunohistochemistry, ×200)

**2.2 各组肺癌A549细胞中MCP-1蛋白表达水平** MCP-1-siRNA实验检测结果显示:与空白组比较,si-NC组A549细胞中MCP-1蛋白表达水平差异无统计学意义( $P>0.05$ )。与si-NC组比较,MCP-1-siRNA-1组和MCP-1-siRNA-2组A549细胞中MCP-1蛋白表达水平均明显降低( $P<0.01$ )。MCP-1过表达实验检测结果显示:与对照组和OE-NC组比较,OE-MCP-1组A549细胞中MCP-1蛋白表达水平明显升高( $P<0.01$ )。见图2和3。

**2.3 各组肺癌A549细胞迁移率** 与si-NC组(23.33%±0.78%)比较,MCP-1-siRNA-1组和MCP-1-siRNA-2组细胞迁移率(11.50%±0.69%和10.51%±1.19%)均明显降低( $P<0.01$ )。见图4。

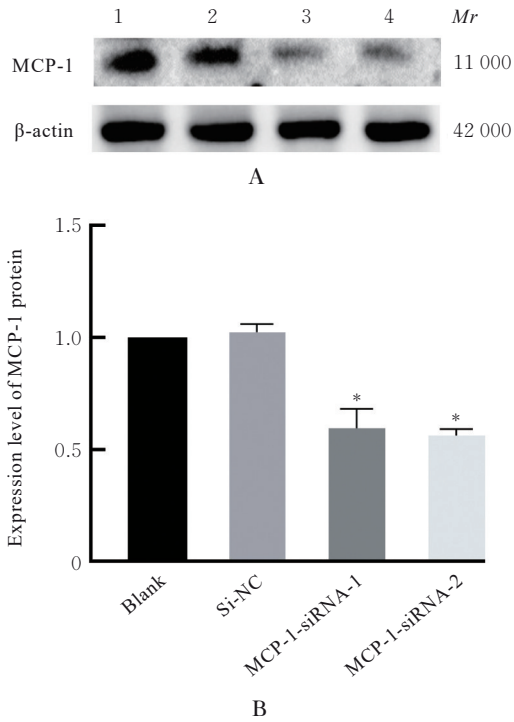
表1 不同临床病理特征NSCLC患者癌组织中MCP-1蛋白表达情况

Tab. 1 Expressions of MCP-1 protein in cancer tissue of NSCLC patients with different clinicopathological characteristics

Clinicopathological characteristic	n	MCP-1		$\chi^2$	P
		-	+		
Gender					
Male	34	15	19	1.105	0.293
Female	46	15	31		
Age					
≤60	20	8	12	0.071	0.790
>60	60	22	38		
Maximum diameter of tumor(cm)					
≤3	53	21	32	0.302	0.583
>3	27	9	18		
Histological type					
Squamous cell carcinoma	21	5	16	2.277	0.131
Adenocarcinoma	59	25	34		
Differentiation					
Well-moderate	53	21	32	0.302	0.583
Poor	27	9	18		
TNM stage					
I	52	24	28	4.747	0.029
II+III	28	6	22		
Lymph node metastasis					
Yes	26	5	21	5.485	0.019
No	54	25	29		

与OE-NC组(8.55%±0.13%)比较,OE-MCP-1组细胞迁移率(24.45%±2.62%)明显升高( $P<0.01$ );与OE-MCP-1组比较,OE-MCP-1+PD98059组细胞迁移率(15.87%±0.71%)明显降低( $P<0.01$ );与OE-MCP-1+PD98059组比较,PD98059组细胞迁移率(4.88%±0.09%)明显降低( $P<0.01$ )。见图5。

**2.4 各组肺癌A549细胞侵袭细胞数** 与si-NC组(274.30个±12.01个)比较,MCP-1-siRNA-1组和MCP-1-siRNA-2组侵袭细胞数[(90.67个±9.29个)和(87.67个±3.06个)]明显减少( $P<0.01$ );与OE-NC组(132.30个±6.51个)比较,OE-MCP-1组侵袭细胞数(295.30个±13.80个)明显增加( $P<0.01$ );与OE-MCP-1组比较,OE-MCP-1+PD98059组侵袭细胞数(181.70个±11.68个)明显减少( $P<0.01$ );与OE-MCP-1+



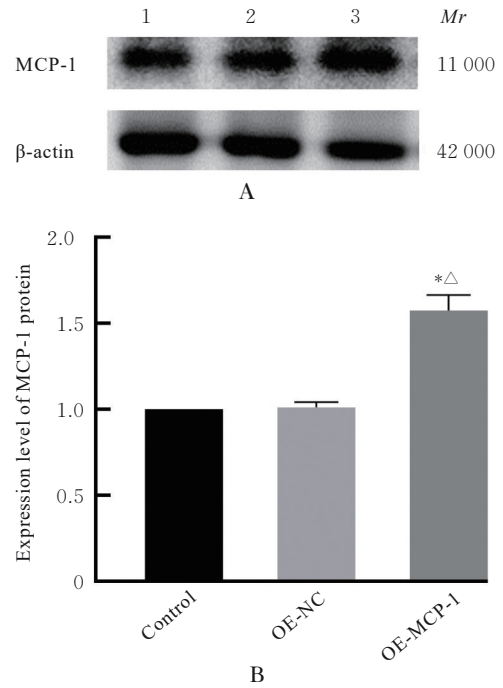
Lane 1: Blank group; Lane 2: si-NC group; Lane 3: MCP-1-siRNA-1 group; Lane 4: MCP-1-siRNA-2 group. \* $P < 0.01$  vs si-NC group.

图2 MCP-1-siRNA 实验中各组肺癌 A549 细胞中 MCP-1 蛋白表达电泳图(A)和直条图(B)

Fig. 2 Electrophoregram (A) and histogram (B) of expressions of MCP-1 protein in lung cancer A549 cells in various groups in MCP-1-siRNA experiment

PD98059 组比较, PD98059 组侵袭细胞数 (66.67 个 ± 6.81 个) 明显减少 ( $P < 0.01$ )。见图 6。

**2.5 各组肺癌 A549 细胞中 p-ERK、t-ERK 和 EMT 相关蛋白表达水平** 与 si-NC 组比较, MCP-1-siRNA-1 组和 MCP-1-siRNA-2 组 A549 细胞中 p-ERK、Vimentin 和 N-cadherin 蛋白表达水平均明显降低 ( $P < 0.05$  或  $P < 0.01$ ), E-cadherin 蛋白表达水平均明显升高 ( $P < 0.01$ )。见图 7。与 OE-NC 组比较, OE-MCP-1 组 A549 细胞中 p-ERK、Vimentin 和 N-cadherin 蛋白表达水平均明显升高 ( $P < 0.01$ ), E-cadherin 蛋白表达水平明显降低 ( $P < 0.01$ )。与 OE-MCP-1 组比较, OE-MCP-1+PD98059 组 A549 细胞中 p-ERK、Vimentin 和 N-cadherin 蛋白表达水平均明显降低 ( $P < 0.01$ ), E-cadherin 蛋白表达水平明显升高 ( $P < 0.05$ )。与 OE-MCP-1+PD98059 组比较, PD98059 组 A549 细胞中 p-ERK、Vimentin 和 N-cadherin 蛋白表达水平均明显降低 ( $P < 0.05$  或  $P < 0.01$ ),



Lane 1: Control group; Lane 2: OE-NC group; Lane 3: OE-MCP-1 group. \* $P < 0.01$  vs control group;  $\Delta P < 0.01$  vs OE-NC group.

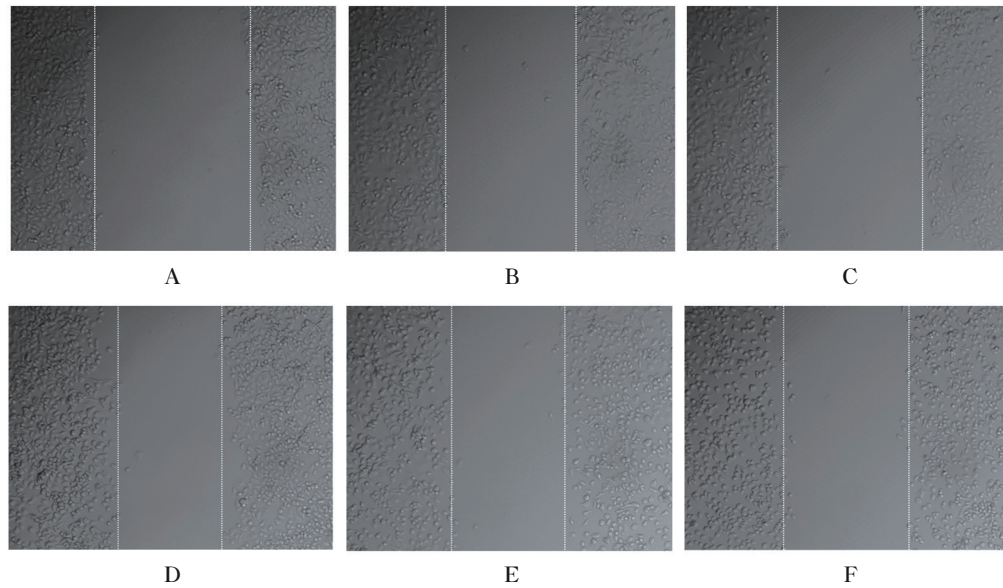
图3 MCP-1 过表达实验中各组肺癌 A549 细胞中 MCP-1 蛋白表达电泳图(A)和直条图(B)

Fig. 3 Electrophoregram (A) and histogram (B) of expressions of MCP-1 protein in lung cancer A549 cells in various groups in MCP-1 over-expression experiment

E-cadherin 蛋白表达水平明显升高 ( $P < 0.01$ )。各组 A549 细胞中 t-ERK 蛋白表达水平比较差异无统计学意义 ( $P > 0.05$ )。见图 8。

### 3 讨论

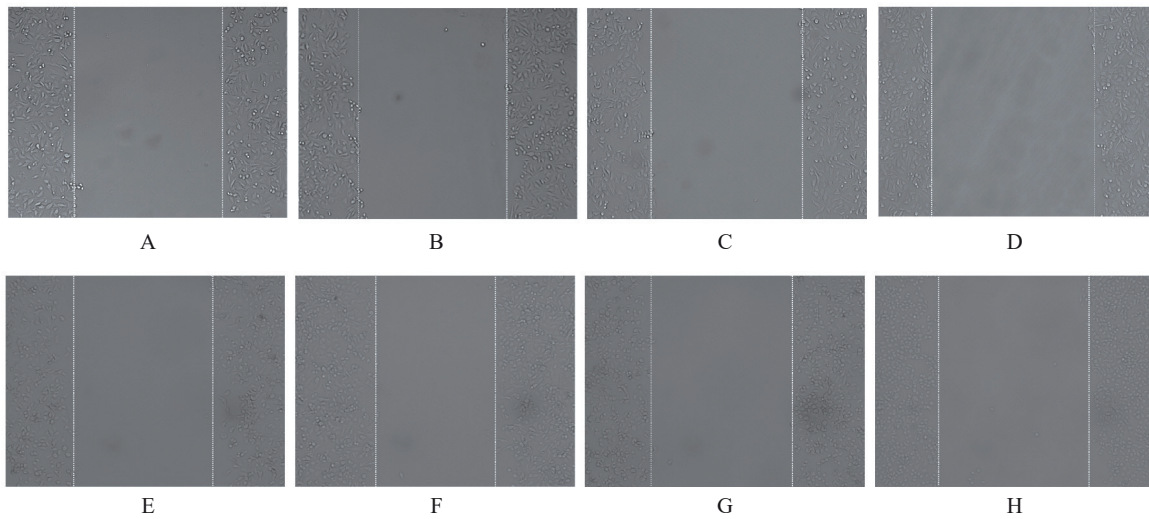
肺癌是全球重大的健康问题,也是癌症导致死亡的重要原因。近年来肺癌的诊断和治疗方面取得了一定进展,但随着肿瘤耐药和转移的发生,肺癌患者的预后仍然较差,需要寻找肺癌治疗的有效靶点。研究<sup>[6-8]</sup>证实:MCP-1在多种肿瘤细胞中高表达,通过影响肿瘤微环境、促进血管生成和诱导 EMT 等过程促进肿瘤转移,发挥促癌作用。血清 MCP-1 水平可作为预测胰腺癌患者预后的潜在标志物,高表达 MCP-1 是淋巴瘤转移及预后不良的危险因素<sup>[9-10]</sup>。此外, MCP-1 还可预测乳腺癌的早期复发<sup>[11]</sup>。本研究结果显示: MCP-1 蛋白在 NSCLC 组织中阳性表达率明显高于癌旁正常肺组织,并与 TNM 分期及淋巴结转移有关,提示 MCP-1 可能促进 NSCLC 细胞的侵袭和转移,与



A—C: 0 h; D—F: 24 h; A, D: Si-NC group; B, E: MCP-1-siRNA-1 group; C, F: MCP-1-siRNA-2 group.

图4 MCP-1-siRNA 实验中各组肺癌 A549 细胞迁移情况( $\times 10$ )

Fig. 4 Migration of lung cancer A549 cells in various groups in MCP-1-siRNA experiment( $\times 10$ )



A—D: 0 h; E—H: 24 h; A, E: OE-NC group; B, F: OE-MCP-1 group; C, G: OE-MCP-1+PD98059 group; D, H: PD98059 group.

图5 MCP-1 过表达实验中各组肺癌 A549 细胞迁移情况( $\times 10$ )

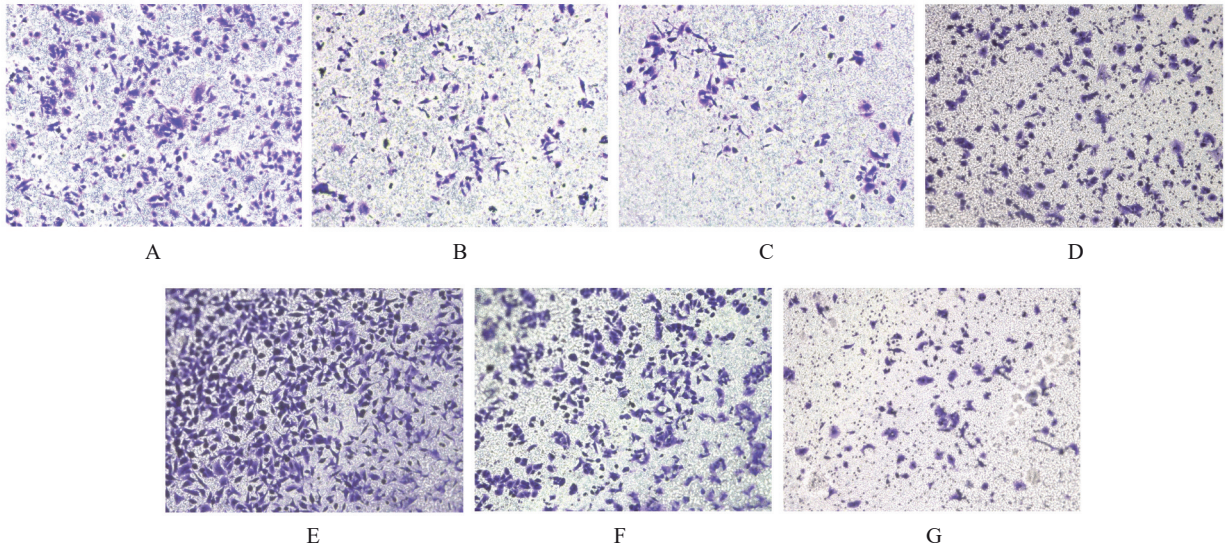
Fig. 5 Migration of lung cancer A549 cells in various groups in MCP-1 over-expression experiment( $\times 10$ )

LI等<sup>[12]</sup>的研究结果一致。

EMT是由上皮细胞来源恶性肿瘤细胞获得迁移和侵袭能力的重要生物学过程。EMT发生过程中,当E-cadherin转化为N-cadherin时,可促进细胞增殖,细胞生长失去控制,增强肿瘤细胞的迁移和侵袭能力,在肿瘤的进展过程中具有重要作用。N-cadherin异常表达常赋予肿瘤细胞由原发肿瘤范围内逃逸并转移至继发部位的能力,使其获得侵袭性的肿瘤表型,是EMT的标志。Vimentin是典型的间充质分子标志物,对患者的治疗及预后评估

具有重要的指导意义<sup>[13-14]</sup>。DONG等<sup>[15]</sup>证实:MCP-1蛋白可通过磷脂酰肌醇3-激酶(phosphatidylinositol 3-kinase, PI3K)/AKT信号通路促进舌癌的侵袭和转移。在口腔鳞状细胞癌中,MCP-1蛋白可通过上调Snail诱导EMT,促进细胞迁移<sup>[16]</sup>。肿瘤相关巨噬细胞通过MCP-1/AKT/ $\beta$ -catenin信号通路传导,促进三阴性乳腺癌的EMT和癌症干细胞特性<sup>[17]</sup>。

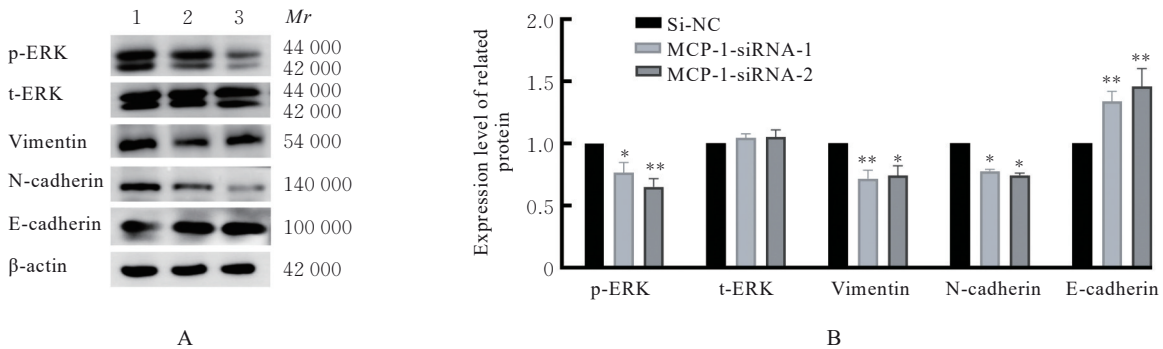
本研究结果显示:沉默肺癌A549细胞MCP-1蛋白表达后,A549细胞迁移率和侵袭细胞数均明



A: Si-NC group; B: MCP-1-siRNA-1 group; C: MCP-1-siRNA-2 group; D: OE-NC group; E: OE-MCP-1 group; H: OE-MCP-1+PD98059 group; G: PD98059 group.

图 6 各组肺癌 A549 细胞侵袭情况(结晶紫, ×10)

Fig. 6 Invasion of lung cancer A549 cells in various groups(Crystal violet, ×10)



Lane 1: Si-NC group; Lane 2: MCP-1-siRNA-1 group; Lane 3: MCP-1-siRNA-2 group. \* $P < 0.05$ , \*\* $P < 0.01$  vs si-NC group.

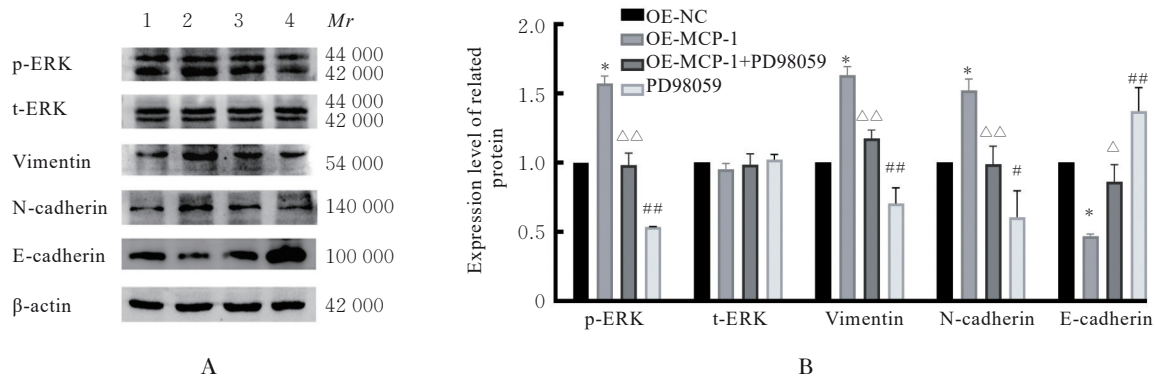
图 7 MCP-1-siRNA 实验中各组肺癌 A549 细胞中 p-ERK、t-ERK 和 EMT 相关蛋白表达电泳图(A)及直条图(B)

Fig. 7 Electrophoregram (A) and histogram (B) of expressions of p-ERK, t-ERK, and EMT-related proteins in lung cancer A549 cells in various groups in MCP-1-siRNA experiment

显减少, EMT 相关蛋白 Vimentin 和 N-cadherin 蛋白表达水平降低, E-cadherin 蛋白表达水平升高。过表达 MCP-1 蛋白可明显促进 A549 细胞迁移和侵袭, 提示 MCP-1 蛋白可明显提高 A549 细胞迁移和侵袭的能力, 促进 EMT 发生。

MCP-1 蛋白可以激活多种信号通路, 促进肿瘤细胞侵袭和转移<sup>[18]</sup>。ERK 信号通路是肿瘤细胞增殖、迁移和凋亡的关键调节剂, 对肿瘤发生发展发挥重要作用。研究<sup>[19]</sup>显示: 高表达早期生长应答因子 1 (early growth response 1, EGR1) 的胃癌 SGC7901R 细胞可激活 MCP-1 蛋白并促进 EMT, MCP-1 蛋白也可激活 ERK 通路, 促进 EGR 活性,

进而形成 EGR1-MCP-1 反馈环路, 促进胃癌细胞发生 EMT 和血管生成。此外, MCP-1 蛋白还可通过 ERK 信号通路促进辐射诱导的肺泡上皮细胞发生 EMT, 在辐射引起的肺毒性作用中发挥关键作用<sup>[20]</sup>。然而, 在肺癌发生发展过程中, MCP-1 蛋白与 ERK 信号通路之间的关系尚未完全阐明。本研究结果显示: 沉默肺癌 A549 细胞 MCP-1 蛋白表达可抑制 p-ERK 蛋白表达, 并抑制细胞迁移和侵袭能力; 过表达 MCP-1 蛋白可促进 p-ERK 蛋白表达水平升高并促进细胞迁移和侵袭; PD98059 可显著抑制过表达 MCP-1 蛋白对肺癌 A549 细胞迁移和侵袭的促进作用, 同时下调 p-ERK 蛋白表达。以



Lane 1: OE-NC group; Lane 2: OE-MCP-1 group; Lane 3: OE-MCP-1+PD98059 group; Lane 4: PD98059 group. \* $P < 0.01$  vs OE-NC group;  $\Delta P < 0.05$ ,  $\Delta\Delta P < 0.01$  vs OE-MCP-1 group; # $P < 0.05$ , ## $P < 0.01$  vs OE-MCP-1+PD98059 group.

图8 MCP-1过表达实验中各组肺癌A549细胞中p-ERK、t-ERK和EMT相关蛋白表达电泳图(A)及直条图(B)

Fig. 8 Electrophoregram (A) and histogram (B) of expressions of p-ERK, t-ERK, and EMT-related proteins in lung cancer A549 cells in various groups in MCP-1 over-expression experiment

上结果均提示: MCP-1蛋白可上调肺癌A549细胞中EMT相关蛋白表达, 促进肺癌A549细胞的迁移和侵袭, 其可能的作用机制与MCP-1蛋白激活ERK信号通路有关。

综上所述, MCP-1蛋白通过激活ERK信号通路, 调控EMT相关蛋白表达, 促进肺癌A549细胞的迁移和侵袭, 本研究结果为肺癌治疗提供了新的方向和靶点。MCP-1蛋白在肿瘤细胞迁移、定植、增殖和侵袭中发挥重要作用, 靶向MCP-1蛋白及其受体的拮抗剂有望成为新开发型抗肿瘤药物的靶向目标。

#### 利益冲突声明:

所有作者声明不存在利益冲突。

#### 作者贡献声明:

王远参与实验操作、数据收集和论文撰写, 王志娟、张明姝、王艺慧和张晴参与实验操作及数据收集, 叶丽平参与实验设计、数据整理和统计分析及论文审校。

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