Experimental study on molecular mechanism of nm23-H1 gene transfection reversing the malignant phenotype of human high-metastatic large cell lung cancer cell line  

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【Abstract】 Background and objective nm23-H1 gene is a well-known tumor metastasis suppression gene. Our previous study has found that transfection of wild type nm23-H1 gene can significantly downregulate the ERK1/2 activity of human high-metastatic large cell lung cancer cell line L9981. The aim of this study is to investigate the influence of nm23-H1 and exogenous ERK1/2 pathway inhibitor U0126 on the extracellular signal-regulated kinase (ERK1/2) of human high-metastatic large cell lung cancer cell line L9981 and its malignant biological behaviors. Methods The expressive levels of total-ERK1/2, dually phosphorylated ERK1/2 and ERK1/2 relative activity of the human high-metastatic large cell lung cancer cell lines, L9981 (parent cell line with nm23-H1 gene hetero-deletion), L9981-nm23-H1 (transfected with nm23-H1 gene) and L9981-PLXSN (transfected with vector) were detected by Western blot and immunoprecipitation technique after treating with U0126 (40 μmol/L for 20 minutes). The in vitro proliferative and invasive abilities among the
above three lung cancer cell lines were determined by MTT and improved Boyden chamber methods. Results
The phosphorylated ERK1/2 expression level and relative activity in L9981-mm23-H1 lung cancer cell line were
remarkably lower than those in L9981 and L9981-PLXSN lung cancer cell lines after being treated with U0126
(P<0.01), but there was no significant difference between L9981 and L9981-PLXSN lung cancer cell lines.
No significant difference of total ERK1/2 expression level was observed among the three lung cancer cell lines
(P>0.05) after being treated with U0126. The in vitro proliferation and invasion of L9981-mm23-H1 lung cancer cell line were remarkably lower than those of L9981 and L9981-PLXSN lung cancer cell lines (P<
0.01), but no significant difference was found between L9981 and L9981-PLXSN lung cancer cell lines (P>
0.05); U0126 could significantly down-regulate the in vitro proliferation and invasion of L9981 lung cancer cell line (P<0.01). Conclusion Blockings the activity of ERK1/2 in L9981 lung cancer cell line and transfecting
the mm23-H1 gene into the L9981 lung cancer cell line may produce similar cell biological behavior changes,
namely the significant reduction of in vitro proliferation and invasion of L9981 lung cancer cell line. These
results indicate that the molecular mechanism which mm23-H1 gene reverses invasion and proliferation of the
human high-metastatic large cell lung cancer cell line may be related to its effects of down-regulating the activity
of the key kinase ERK1/2 of Ras-to-MAPK signal transduction pathway.

【Key words】 Human high-metastatic lung cancer cell line mm23-H1 gene ERK1/2
Proliferation Invasion
This work was supported by a grant from the Key Project of National Natural Science Foundation of China
(to ZHOU Qinghua) (No. 30430300).

nm23-H1 ； ECM ； Sigma ； PVDF ； Millipore ；

1.3 ； 70% ～ 80% ； 40 μmol/L ； DMSO ； DMSO ； (20 mmol/L Tris-HCl pH7.5,
150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1
mmol/L β-glycerophosphate, 1 mmol/L Na3VO4, 1

1.4 Western blot ； ERK1/2 ； ERK1/2/ERK1/2 phosphorylation state independent

1.2 p44/42 MAP kinase antibody kit
Bio-rad 2000 系统用于 X 线胶片的洗膜和碘化液的定影，IOD 标准曲线用于该小室法测定细胞体外侵袭能力。

以上操作均在冰上进行，终止反应的酶缓冲液和裂解液均需冻存备用。


center-align

1.5 ERK1/2 与 Phospho-p42/44 MAPK 选择对数生长期的细胞用无血清混合液培养基中培养，弃培养基，加入相应处理因素，4°C 15 μL 0.1 ml/mL，3 复孔，100°C 5 min，25 μL 3× SDS，1% Triton X-100，30 min，25 μL 3× SDS 2 min，100°C 20 h，裂解细胞，加入化学发光法同上，重悬的固定化细胞株磷酸化表达水平均显著低于不同浓度的 DMSO 处理组，而处理组与对照组之间表达水平无统计学差异。不同浓度的 DMSO 处理组处理组间磷酸化表达水平的差异，以方差分析法检验 (P = 0.00)；ERK1/2 与 DMSO 各浓度组处理组间磷酸化表达水平的差异，以独立样本 t 检验分析 (P = 0.485) (r = 1)。

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2.1 U0126 对 ERK1/2 与 Phospho-p42/44 MAPK 的抑制作用，采用 DMSO，L9891，L9891-PLXSN，L9891-mm23-H1，ERK1/2 处理组，ERK1/2 处理组和 DMSO 各浓度组处理组间磷酸化表达水平的差异，均以方差分析法检验 (P = 0.00)；ERK1/2 处理组与 DMSO 各浓度组处理组间磷酸化表达水平的差异，以独立样本 t 检验分析 (P = 0.05)。

图 1 比较 ERK1/2，L9891，L9891-PLXSN，L9891-mm23-H1，U0126 处理组及 DMSO 各浓度组处理组间磷酸化表达水平的差异，均以方差分析法检验 (P = 0.00)；ERK1/2 处理组与 DMSO 各浓度组处理组间磷酸化表达水平的差异，以独立样本 t 检验分析 (P > 0.05)。

2.2 U0126，L9891，L9891-PLXSN，L9891-mm23-H1，ERK1/2 处理组，DMSO 各浓度组处理组间磷酸化表达水平的差异，均以方差分析法检验 (P = 0.00)；ERK1/2 处理组与 DMSO 各浓度组处理组间磷酸化表达水平的差异，以独立样本 t 检验分析 (P = 0.05)。
图1 U0126（\(\mu\)mol/L）对磷酸化 Elk-1的影响

**Tab 1** Comparison of in vitro invasion in different human high-metastatic large cell lung cancer cell lines treated with U0126

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Numbers of invasive cells (x±s)</th>
<th>Control (0 (\mu)mol/L)</th>
<th>Test (60 (\mu)mol/L)</th>
<th>(P) value</th>
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</thead>
<tbody>
<tr>
<td>L9891</td>
<td>170.0±7.28</td>
<td>70.6±12.55</td>
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</tr>
<tr>
<td>L9891-PLXSN</td>
<td>168.6±7.16</td>
<td>67.0±9.57</td>
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<tr>
<td>L9891-nm23-H1</td>
<td>59.8±8.35</td>
<td>14.6±5.59</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>(P) value</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
</tbody>
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**图2** 比较不同条件下的细胞体外增殖活性

图3 比较不同条件下的细胞体外侵袭能力
nm23-H1
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<table>
<thead>
<tr>
<th>Steeg</th>
<th>MDA-MB-435</th>
<th>nm23-H1</th>
<th>KSR</th>
</tr>
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<tr>
<td>Ras-1</td>
<td>KSR</td>
<td></td>
<td>KSR</td>
</tr>
<tr>
<td></td>
<td>RaI, MEK, ERK</td>
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<tr>
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<td>MAPK</td>
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<td>KSR</td>
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<td>KSR→ERK</td>
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