nm23-H1  L9981

Molecular Mechanism of Reversing Metastatic Phenotype in Human
High-metastatic Large Cell Lung Cancer Cell Line L9981 by nm23-H1
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LIU Lun-Xu, CHEN Xiao-He, SUN Yi-Lin, SUN Ze-Fang

[ABSTRACT] BACKGROUND & OBJECTIVE: nm23-H1, a tumor metastasis suppressive gene, can reverse tumor metastasis phenotype. But the molecular mechanism of nm23-H1 in inhibiting or reversing metastasis of lung cancer is unclear. This study was to explore the molecular mechanism of nm23-H1 in reversing metastasis phenotype of lung cancer. METHODS: nm23-H1 gene and pLXSN were seperately transfected into human lung cancer cell line L9981. Proliferation of L9981, L9981-pLXSN, and L9981-nm23-H1 cells was detected by MTT assay, cell invasive ability was detected by modified Boyden chamber. Tumorigenesis and experimental lung metastasis were determined in vivo. mRNA and protein levels of &beta;catenin, E-Cadherin, CD44S, CD44V6, matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-1 (TIMP-1), and vascular endothelial growth factor (VEGF) were detected by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot. RESULTS: (1) Cell proliferation, clone formation, and invasive ability were significantly lower in L9981-nm23-H1 cells than in L9981 cells [(19.5±2.9)% vs. 100%, 10.3±0.7 vs. 21.7±1.3, 31.0± 3.0 vs. 151.0±6.3, P<0.01]. (2) The inhibitory rate of tumorigenesis of nude mice was significantly higher in L8981-nm23-H1 group than in L9981 group (85.6% vs. 0%, P<0.001); the lung metastatic rate was significantly lower in L9981-nm23-H1 group than in L9981 group (0% vs. 100%, P<0.001). (3) nm23-H1 up-regulated mRNA and protein levels of &beta;catenin, E-Cadherin, and TIMP-1, and down-regulated levels of MMP-2, CD44V6, and VEGF (P<0.01). (4) nm23-H1 up-regulated mRNA level of CD44s, protein level of CD44s didn't change (P>0.05). CONCLUSION: nm23-H1 gene can reverse malignant and metastatic phenotype of L9981 cells through regulating the expressions of lung cancer metastasis-related genes.

KEYWORDS: nm23-H1 gene; Metastatic phenotype; Metastasis-related genes; Lung neoplasms
材料与方法

1.1

1.1.1 L9891

1.1.2

PCR

1.1.3

Table 1 Primers of nm23-H1 and metastasis-related genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-catenin</td>
<td>S, 5'-TCTCGTCTGGCTACTGC-3'; AS, 5'-CAGTCTCGGTCACTGGC-3'</td>
<td>300</td>
</tr>
<tr>
<td>VEGF</td>
<td>S, 5'-TTGCGCTGGCTACTGC-3'; AS, 5'-CAGTCTCGGTCACTGGC-3'</td>
<td>300</td>
</tr>
<tr>
<td>CD44S</td>
<td>S, 5'-CTCGTCTGGCTACTGC-3'; AS, 5'-CAGTCTCGGTCACTGGC-3'</td>
<td>300</td>
</tr>
<tr>
<td>MMP-2</td>
<td>S, 5'-ACGACGTGGCTGGCAGGCACG-3'; AS, 5'-CAGTCTCGGTCACTGGC-3'</td>
<td>300</td>
</tr>
<tr>
<td>CD44V6</td>
<td>S, 5'-CTCGTCTGGCTACTGC-3'; AS, 5'-CAGTCTCGGTCACTGGC-3'</td>
<td>300</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>S, 5'-AGCGATGAGTGGCTGGCAGGCACG-3'; AS, 5'-CAGTCTCGGTCACTGGC-3'</td>
<td>300</td>
</tr>
<tr>
<td>β-actin</td>
<td>S, 5'-GACTACGTGGTGCTGGCAGACG-3'; AS, 5'-AGCGATGAGTGGCTGGCAGGCACG-3'</td>
<td>500</td>
</tr>
</tbody>
</table>


1.2

1.2.1 RPMI-1640

1.2.2 (MTT)
1.2.3 Treatment of samples (Boyden chamber) .

1.2.5 RNA extraction .

1.2.6 RT-PCR .

1.2.7 Western blot .

1.2.8 Real-time PCR .

1.2.9 RT-PCR .

2.1 nm23-H1 group 

2.2 L9981 group 

2.3 nm23-H1 group

Figure 1 Growth curves of L9981, L9981-pLXSN, and L9981-nm23-H1 cells detected by MTT assay
Figure 2 Invasive abilities of L9981, L9981-pLXSN, and L9981-nm23-H1 cells detected by modified Boyden chamber methods (HE ×175)

A, L9981 cells; B, L9981-pLXSN cells; C, L9981-nm23-H1 cells.

Table 2 Suppression of tumorigenesis of L9981 cells in nude mice by nm23-H1

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumorgenesis</th>
<th>Weight of tumor (g, <em>x̄</em>)</th>
<th>Inhibitory rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L9981</td>
<td>7</td>
<td>100.0</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>L9981-pLXSN</td>
<td>7</td>
<td>100.0</td>
<td>3.2±0.6</td>
</tr>
<tr>
<td>L9981-nm23-H1</td>
<td>4</td>
<td>57.1</td>
<td>0.6±0.3</td>
</tr>
</tbody>
</table>

Each group contains 7 mice.

2.6 L9981 nm23-H1 mRNA expression

L9981-nm23-H1 β-catenin, E-Cadherin, TIMP-1, CD44S (P<0.05), L9981-pLXSN (P<0.05).
2.7 L9981 nm23-H1

400~500 mg/L

SDS-PAGE

90%

L9981-nm23-H1 β-catenin, E-Cadherin, TIMP-1, VEGF, CD44V6

(P<0.01), CD44s (P>0.05) (4)

nm23-H1 eDNA

26.67%, L9981-nm23-H1 (6,7)

L9981

nm23-H1 eDNA

L9981 nm23-H1 (3-5)

nm23-H1

nm23-H1 eDNA

nm23-H1 eDNA

nm23-H1 eDNA
功能蛋白转染可以显著降低细胞的转录降低能阻断肿瘤相关基因的变化对三种低表达基因分别转入具有低转移和高转移潜能细胞。

术研究发现，信号传导通路中磷酸化游调控基因与细胞之间的粘附。

我们应用基因芯片技术检测基因的异常导致一些肿瘤最近研究发现，基因抑制肿瘤转移的分子机制仍不清，但最近发现基因转染肿瘤细胞后可以逆转肿瘤细胞的恶性。

这些研究结果提示，这些基因在不同组织和细胞的表达具有不同的同的生理功能。

信号传导和转录因子等，基因的表达无影响。

而且基因抑制肿瘤转移的功能与基因的表达以抑制其在不同组织和细胞的表达。

总之，癌细胞的转移表型的分子机制。

在本实验中观察到这些研究结果提示，这些基因在不同组织和细胞的表达具有不同的同的生理功能。

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