Establishment and their biological characteristics of clonal cell subpopulations NL9980 and L9981 from a human lung large cell carcinoma cell line WCQH-9801

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Abstract

Objective To explore the possibility of separating and establishing clonal cell subpopulations with different metastatic phenotype from a human lung large cell carcinoma cell line WCQH-9801 to identify the difference of biological and molecular biology between NL9980 and L9981 cell lines. Methods Two sub-cell lines NL9980 and L9981 were isolated and established from a human lung large cell carcinoma cell line WCQH-9801 by the single cell cloning techniques. The RELP mRNA and protein transcript expression were detected in NL9980 and L9981 cell lines by Southern blot RT-PCR and Western blot. The biological characteristics of *vivo* and *vitro* were determined in NL9980 and L9981 cell lines by MTT plate Boyden chamber methods and animal models. Results 1 Two sub-cell lines NL9980 and L9981 which had different metastatic phenotype were successfully isolated and established from a human lung large cell carcinoma cell line WCQH-9801. 2 The L9981 cell line had LOH of nm23-H1 gene deletion of mRNA and protein expression of nm23-H1 but the NL9980 cell line had neither LOH of nm23-H1 nor deletion of mRNA and protein expression of nm23-H1. 3 The proliferation clone formation and *vitro* invasion of L9981 cell line were significantly higher than those of NL9980 cell line. 4 The tumorigenicity and lung metastatic rate in nude mouse of L9981 cell line were remarkably higher than those of NL9980. 5 No significant difference of the chromosome number was observed between NL9980 and L9981 cell lines. Conclusion 1 NL9980 and L9981 cell lines established from a human lung large cell carcinoma cell line have different biological and molecular characteristics. 2 The high invasio and metastasis ability of L9981 cell line might be related to the LOH of nm23-H1 gene.

Key words Human lung large cell carcinoma Invasion and metastasis Cloning cell line NL9980 and L9981 Tumorigenicity

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材料与方法

1.1 变、侵袭转移、多药耐药的分子机理、生物学特征,以及基因(上游引物:WG
小牛血清,产物

1.2 nm23-H1 2 - 40 m 5 - 96

1.3 Southern 变、侵袭转移、多药耐药的分子机理、生物学特征,以及基因(上游引物:WG

1 EcoR I L 2 - 40 m 5 - 96

1.4 RT-PCR M-MLV 变、侵袭转移、多药耐药的分子机理、生物学特征,以及基因(上游引物:WG

1.5 Western Blot 变、侵袭转移、多药耐药的分子机理、生物学特征,以及基因(上游引物:WG

1.6 MTT

1.7

1.8

1.9

1.10

1.11

2
因多态性比较

基因在细胞株中以杂合子（或及）的形式存在，而细胞株中和均缺失，因此为杂合缺失（图1）。

图1 人肺癌细胞株和细胞株中基因限制性片段长度多态性

图2 人肺癌细胞株和细胞株中基因转录表达比较

测定细胞株蛋白提取液中蛋白含量为。杂交后的结果显示，细胞株中蛋白表达是完全缺失，而同一来源的的蛋白表达正常（图3）。

图3 人肺癌细胞株和细胞株中基因转录表达电泳图

体外生长曲线比较

细胞株增殖水平在第、、天均显著高于株（），而第、天和细胞株增殖水平均无显著性差异（）（图4）。

图4 人肺癌细胞株和细胞株生长曲线图

克隆形成能力的比较

采用平板法检测和细胞的克隆形成能力，结果显示：细胞克隆形成率显著高于细胞（）（表1）。

采用改良的X7PI;小室法检测和细胞的体外侵袭力，结果显示出显著差异（）。

表1 比较

<table>
<thead>
<tr>
<th>细胞株</th>
<th>克隆形成率</th>
<th>P值</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9981</td>
<td>21.67 ± 1.33</td>
<td>0.001</td>
</tr>
<tr>
<td>NL9980</td>
<td>7.033 ± 1.67</td>
<td></td>
</tr>
</tbody>
</table>

2.6 细胞株和细胞株的体外侵袭力

2.7 细胞株和细胞株的体外侵袭力
Cellular invasive ability across the extracellular matrix, the results show:

- Cell line L9981 invasion force significantly higher than cell line NL9980 (Fig. 6).

### Table 2 Comparison of cell extracellular invasion between L9981 and NL9980 cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cell No. of invasion $\bar{x} \pm s$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L9981</td>
<td>157.00 ± 6.33</td>
<td>0.000</td>
</tr>
<tr>
<td>NL9980</td>
<td>37.00 ± 1.33</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 Comparison of tumorigenicity in nude mice between L9981 and NL9980 cell lines

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>L9981 $\bar{x} \pm s$</th>
<th>NL9980 $\bar{x} \pm s$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td>6</td>
<td>30.25 ± 1.08</td>
<td>29.27 ± 1.51</td>
<td>0.721</td>
</tr>
<tr>
<td>Tumor</td>
<td>6</td>
<td>3.67 ± 1.01</td>
<td>0.239 ± 0.57</td>
<td>0.023</td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
<td>1.160 ± 0.25</td>
<td>1.18 ± 0.31</td>
<td>0.814</td>
</tr>
</tbody>
</table>

### Table 4 Comparison of the lung metastasis in nude mice between L9981 and NL9980 cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>No. of metastasis in lung</th>
<th>Mean $\bar{x} \pm s$</th>
<th>Metastatic rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>L9981</td>
<td>1  2  3  4  5  6</td>
<td>2.68 ± 1.63</td>
<td>100.0</td>
</tr>
<tr>
<td>NL9980</td>
<td>0  0  0  1  0  0</td>
<td>0.17 ± 0.41</td>
<td>16.67</td>
</tr>
<tr>
<td>$P$ value</td>
<td></td>
<td>0.005</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**Discussion**

This study utilizes single cell cloning technology to successfully establish two lung cancer cell lines, L9981 and NL9980, from the maternal cell line R1. The invasive and metastatic abilities, as well as molecular characteristics, of these two cell lines further demonstrate the heterogeneity in malignancy and metastasis among different lung cancer cell lines. This suggests the presence of different biological and molecular subtypes within the same tumor cell line.
显示: 光镜下表达正常。因此, 本研究从等位基因的杂合性缺失, 并且差异性或异质性决定其具有不同的侵袭转移潜能。本缺失率等位基因缺失率周清华等。人肺癌细胞株其生物学特性有很大不同, 尤其是条带同时存在的个体称为杂合子。人肺鳞癌细胞系和细胞株、远处转移者细胞与母系细胞株仍然具有高。

**参考资料**

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