Transfection of tumor metastasis suppressor gene nm23-H1 can up-regulate the activity of GSK-3β in human high-metastasis large cell lung cancer cell line L9981  

FU Junke1, ZHOU Qinghua2, ZHU Wen1, WANG Yanping1, CHEN Xiaohui1, CHE Guowei1, NIE Qiang1, LI Dingbiao1, LIU Lianxu1, LI Yin1. Key Laboratory of Lung Cancer Molecular Biology of Sichuan Province and Department of Thoracic Surgery, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, P. R. China.

Corresponding author: ZHOU Qinghua. E-mail: zhouqh@mail.sc.cninfo.net

Objective To investigate the influence of tumor metastasis suppressor gene nm23-H1 on the activity of glycogen synthase kinase 3β GSK-3β in human high-metastasis large cell lung cancer cell line L9981.

Methods The levels of GSK-3β expression in cytoplasm and nucleus were determined with anti-GSK-3β antibody in human high-metastasis large cell lung cancer cell line L9981 cell line with nm23-H1 gene dele-
The expression intensity of GSK-3β of cyttoplasm and nucleus was $6.34 \pm 5.41 \times 10^5$ and $4.35 \pm 4.90 \times 10^5$ IOD in L9981-nm23-H1 and L9981-pLXSN respectively. A high significance in GSK-3β expression intensity of both cyttoplasm and nucleus existed among L9981-nm23-H1, L9981-pLXSN and L9981. A multiple comparison showed a highly significant difference was observed when L9981-nm23-H1 was compared with L9981-pLXSN or L9981 $P < 0.01$ but no significant difference was observed between L9981-pLXSN and L9981 $P > 0.05$. The GSK-3β activity in L9981-nm23-H1 was significantly higher than that in L9981-pLXSN and L9981 $P < 0.01$ but no significant difference was observed between the L9981-pLXSN and L9981 $P > 0.05$. After treatment with 20 mmol/L LiCl, the expression intensity of GSK-3β of cyttoplasm and nucleus was $4.71 \pm 4.59 \times 10^5$ and $3.82 \pm 3.50 \times 10^5$ IOD in L9981-nm23-H1 and L9981-pLXSN respectively. A high significant difference in GSK-3β expression existed before and after treatment with LiCl in L9981-nm23-H1 $P > 0.05$. However, the GSK-3β expression intensity in cyttoplasm and nucleus before treatment was remarkably higher than those after treatment in both L9981-pLXSN and L9981 $P < 0.05$. After treatment with 20 mmol/L LiCl, the GSK-3β activity in cyttoplasm and nucleus was $1.09 \pm 1.12 \times 10^5$ and $3.74 \pm 2.15 \times 10^5$ IOD in L9981-nm23-H1 and L9981-pLXSN respectively. The GSK-3β activity both in cyttoplasm and nucleus after treatment with LiCl was remarkably lower than that before treatment in L9981-nm23-H1 and L9981-pLXSN and L9981 $P < 0.01$ or $P < 0.05$. Transfection of nm23-H1 gene can significantly up-regulate the expression level and activity of GSK-3β in human high-metastasis large cell lung cancer cell line L9981. LiCl can remarkably suppress the upregulation of nm23-H1 gene on GSK-3β activity in L9981 cell line. The effects of nm23-H1 gene on suppressing the signal transduction of Wnt pathway might be carried out through upregulating GSK-3β expression and activity in human high-metastasis large cell lung cancer cell line L9981.

Key words Human high-metastasis large cell lung cancer cell line L9981, nm23-H1, Wnt signal pathway, GSK-3β.

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1.2.1 L9981-pLXSN

1.2.3 Western blot

1.2.4 GSK-3β

1.2.4.1 GSK-3β

2.1 L9981-mm23-H1

2.5 L9981-pLXSN

2.6 GSK-3β

Fig 1 The expression of GSK-3β among L9981-pLXSN and L9981-mm23-H1 cell lines by Western blot
Fig 2  Comparison of GSK-3β kinase activity among L9981, L9981-pLXSN and L9981-nm23-H1 cell lines

2.3 20 mmol/L LiCl  GSK-3β  GSK-3β  2 164 ± 151 224 ± 19 IOD  GSK-3β  2 030 ± 155 217 ± 15 IOD  GSK-3β  4 718 ± 549 3 823 ± 350 IOD  P > 0.05

Fig 3  Expression of GSK-3β among L9981, L9981-pLXSN and L9981-nm23-H1 cell lines after co-incubated with 20 mmol/L LiCl

2.4 20 mmol/L LiCl  GSK-3β  GSK-3β  4 435 ± 427 909 ± 156 CPM  L9981-nm23-H1  1 1099 ± 1 1122 3 748 ± 215 CPM
抑制nm23-H1基因活性, 直接或间接地抑制肺癌细胞株的增殖。活化因子和细胞核因子, 热休克因子的主要作用是上调细胞核内磷酸化酶的活性, 从而可能抑制nm23-H1的活性。}

研究发现在人神经纤维瘤细胞中, nm23-H1蛋白的表达无明显减少, 而暴露于无血清培养基、热休克因子或癌基因激活制剂, 能抑制nm23-H1的表达水平和酶活性, 如p53。前期显著降低(p < 0.01)

在转基因细胞株中, nm23-H1, GSK-3β的蛋白表达水平和酶活性均显著高于原代细胞。综合以上研究, 在转基因细胞株中, nm23-H1, GSK-3β的蛋白表达水平和酶活性均显著高于原代细胞。

此外, 本研究还发现nm23-H1, GSK-3β的蛋白表达水平和酶活性与肿瘤转移抑制基因的功能有关。以往人们一直认为nm23-H1, GSK-3β的蛋白表达水平和酶活性与肿瘤转移抑制基因的功能有关。