

Construction of the suppression subtractive cDNA libraries of human large cell lung cancer line L9981 before and after transfection with nm23-H1 gene

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摘要

Background and objective It has been proven that nm23-H1 gene is an important metastatic-suppressed gene of lung cancer. In order to screen the differential expression genes related to nm23-H1, we constructed the suppression subtractive cDNA libraries of human large cell lung cancer line L9981 transfected and untransfected with nm23-H1 gene by suppression subtractive hybridization (SSH) in this study, which lay a solid foundation for further screening and cloning metastatic-related genes of nm23-H1. **Methods** The forward and reverse suppression subtractive cDNA libraries were constructed in the human large cell lung cancer line L9981 before and after transfection with nm23-H1 gene (L9981 and L9981-nm23-H1) by SSH method. The positive clones were preliminarily screened by blue-white colony, and precisely identified by PCR. **Results** The suppression subtractive cDNA libraries were successfully constructed in the human large cell lung cancer line L9981 transfected and untransfected with nm23-H1 gene (L9981-nm23-H1 and L9981). After the blue-white screening, about three hundred positive clones in the forward subtracted library and four hundred positive clones in the reverse subtracted library were obtained. Random analysis of 96 clones in each library with colony PCR methods showed that 84 clones in the forward subtracted library and 83 clones in the reverse subtracted library contained (300-750) bp inserts. **Conclusion** SSH is proved to be an efficient tool for differential expression gene cloning. The forward and reverse suppression subtractive cDNA libraries of human large cell lung cancer line L9981 transfected and untransfected with nm23-H1 gene (L9981-nm23-H1 and L9981) are successfully constructed by SSH and T/A cloning technology. The expression of nm23-H1 gene in the human large cell lung cancer cell lines may affect the differential expression of some metastatic-related genes.






关键词

Lung neoplasms; nm23-H1 gene; Suppression subtractive hybridization; cDNA library; Metastatic-related gene


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


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