Clinical Observations

NORTHERN BLOT ANALYSIS OF nm23 GENE EXPRESSION IN HUMAN LUNG CANCER

LIU Lun-xu 刘伦旭 [1], ZHOU Qing-hua 周清华 [1] SHI Ying-kang 石应康 [1]
QIN Yang 覃扬 [2], SUN Zhi-lin 孙芝琳 [2], SUN Ze-fang 孙泽芳 [2]

[1]Department of Thoracocardiac Surgery, The First University Hospital; [2]Department of Biochemistry, School of Basic Medicine; West China University of Medical Sciences, Chengdu 610041, China

ABSTRACT

Objective: To investigate the role of nm23 gene expression in human lung cancer. Methods: Forty human lung cancer tissues and 19 non-cancer pulmonary tissues were studied for their nm23-H_1 and nm23-H_2 mRNA expression with non-radioactive Northern blot hybridization. The correlation of nm23 mRNA expression with clinical features of lung cancer was analyzed. Results: The mRNA expression of nm23-H_2 gene in poorly differentiated squamous cell carcinoma was significantly decreased compared to that in moderate-high differentiated squamous cell carcinoma. The mRNA expression of nm23-H_1 and nm23-H_2 gene in small cell lung cancer was significantly decreased compared to that in squamous cell carcinoma. No significant difference in nm23 mRNA expression was observed between lung cancer with and without lymph node metastasis, nor was there significant difference between tumor stage. Conclusion: The mRNA expression of nm23 gene is correlated with the degree of differentiation of lung cancer, but there is no evidence of metastasis suppression effect by nm23 gene.

Key words: Lung neoplasms, nm23, Gene expression, RNA, Northern blotting.

The nm23 gene was originally identified by Steeg and colleagues[1] at the National Cancer Institute (USA) in 1988. Two isotypes of nm23 gene in human, known as nm23-H_1 and nm23-H_2[2] have been described, which are both located on chromosome17q21.3 and encode for nucleoside diphosphate kinase (NDPK).[3] The early studies on cancer cell lines revealed that nm23 gene could suppress the motility and metastatic potential of cancer cells, and was proposed to be a metastasis suppressor gene. Moreover, the association of reduced nm23 expression with high metastatic potential has also been documented in several human malignant tumors including breast carcinoma, hepatocarcinoma, gastric carcinoma, malignant melanoma and ovary carcinoma. However, studies on neuroblastoma, thyroid carcinoma and prostate carcinoma failed to prove its antimetastatic activity, but rather showed correlation with tumor proliferation and invasion.[4] In the present investigation, we examined nm23-H_1 and nm23-H_2 gene mRNA expression of human lung cancer by Northern blot hybridization and analyzed its correlation with tumor clinical characteristics.

MATERIALS AND METHODS

Tissue Specimens

Forty primary pulmonary cancer tissues and 19 non-cancer pulmonary tissues distant to cancer foci were obtained during surgery from the Department of Cardiothoracic Surgery of our hospital. 40 lung cancers included 4 small cell pulmonary carcinomas (2 stage I and II, 2 stage III and IV), 18 squamous cell carcinomas (10 poor differentiation, 8 moderate-high differentiation; 7 stage I and II, 11 stage III and IV), 8 adenocarcinomas (5 poor differentiation, 13 moderate-high differentiation; 3 stage I and II, 15 stage III and IV). Among those, 22

Accepted for publication: April 29, 1999
This work was supported in part by a grant from the National Natural Sciences Foundation of China (to ZHOU Qing-hua) (No. 39470687) and a grant from the Chinese Medical Board of New York (to ZHOU Qing-hua) (Y9316).
Correpondence to: ZHOU Qing-hua; Department of Thoracocardiac Surgery, The First University Hospital, West China University of Medical Sciences, No. 37 Guo Xue Xiang, Chengdu 610041, China; Fax: (0086-28)-5560777; E-mail: Zhouqh@mail.sc.cninfo.net.

developed lymph node metastasis. The clinical staging was based on the staging criteria revised by UICC in 1986.

Preparation of Probes

The human Pnm23-H_1 and Pnm23-H_2 cDNA recombinant plasmids were kindly donated by Dr. Patricia S Steeg of NCI and Pr-actin by Prof. SUN Zhilin of our university. Pnm23-H_1, Pnm23-H_2 and Pr-actin were digested respectively with *Bam*H_1, *Eco*RI and *Hind*III/Pst I, which resulted in corresponding 900 bp, 700 bp and 900 bp cDNA insert fragments. The cDNA inserts were recollected by glass powder DNA isolation method, and labeled with non-radioactive digoxigenin using Boehringer Mannheim's random primer DNA labeling kit.

Northern Blot Hybridization

Total RNA of pulmonary tissues was extracted by one-step method of guanidinium thiocyanate-phenol-chloroform. Twenty-five μg of total RNA was fractionated on 1% agarose-formaldehyde gel and blotted onto nylon membrane (Bio-Rad) by capillary transfer. The hybridization was carried out in a solution containing 7% SDS, 50% formamide, 5×SSC, 2% blocking reagent, 50 mmol/L sodium phosphate pH 7.0 and 0.1% sodium dodecylsulfate, and also 30 ng/ml probes. The hybridization and its signal chemiluminescent imaging were performed according to the manufacturer's (Boehringer Mannheim) manual. Every blotted membrane was hybridized with nm23-H_1, nm23-H_2 and r-actin cDNA probes sequentially.

Measurement of Expression Intensity

The intensity of the hybridization signal was scanned and quantitated by UVP GDS-8000. r-actin was employed as an internal control to correct for the uneven amount of RNA loaded in each lane, due to its stable expression in various kind of tissues. The relative expression level of nm23 gene of a certain sample was calculated according to the following equation:

$$\text{relative level of nm23 expression of certain sample} = \frac{\text{scanning intensity of nm23 of certain sample}}{\text{scanning intensity actin of certain sample}} \times \text{average scanning intensity of actin of all samples}$$

Statistical Analysis

The data of this study was quantitative. T test and Wilcoxon test were used. A P value less than 0.05 was considered significant.

RESULTS

nm23-H_1 and nm23-H_2 mRNA Expression in Human Lung Cancer

The 0.8-kilobase transcripts of nm23-H_1 and nm23-H_2 mRNA were expressed in all examined tissues after hybridization with corresponding cDNA probes (Figure 1, the molecular marker was not shown), no altered mRNA transcript or homogenous deletion was detected. There was no significant difference in mRNA expression of nm23H_1 and nm23-H_2 between lung cancer tissues and non-cancer pulmonary tissues (P>0.05, Table 1).

Table 1. Relative expression of nm23 gene mRNA in lung cancer tissues and non-cancer pulmonary tissues

Tissues	n	nm23-H1		nm23-H2	
		$\bar{x}\pm s$	P	$\bar{x}\pm s$	P
Lung cancer tissues	40	42.21±11.71	>0.05	35.01±13.11	>0.05
Non-cancer pulmonary tissues	19	44.78±14.16		35.89±14.23	

Correlation of nm23 mRNA Expression with Clinical Characteristics of Lung Cancer

Referring to the differentiation of pulmonary squmaous cell carcinoma, nm23-H2 mRNA expression in poorly differentiated tumor was significantly decreased compared to that in moderate-high differentiated one (P<0.01). The mRNA expression of nm23-H_1 and nm23-H_2 in small cell lung cancer was significantly lower than that in squamous cell carcinoma (P<0.05). But no significant difference in both nm23-H_1 and nm23-H_2 mRNA expression was observed between lung cancer with and without lymph node metastasis, nor was there significant difference between tumor stage (P>0.05, Table 2).

DISCUSSION

Many studies on nm23 genes have been reported since its original identification. Studies on several tumor cell lines showed clear evidences of correlation of nm23 gene with metastasis suppression.[5] But widely done research on various clinical tumors has provided conflicting results, some were in agreement, and others were inconsistent.

Table 2. Correlation of nm23 mRNA expression with clinical characteristics of lung cancer

Clinical characteristics	n	nm23-H_1		nm23-H_2	
		$\bar{x}\pm s$	P	$\bar{x}\pm s$	P
Lung cancer					
①Undifferentiated	4	31.20±14.52	>0.05*	23.82±7.61	>0.05*
②Poorly differentiated	15	44.92±11.85		35.60±10.98	
③Moderate-high differentiated	21	42.36±10.37		36.73±14.61	
	12	43.07±12.85	>0.05	32.06±11.17	>0.05
Stage I and II					
Stage III and IV	28	41.84±11.41		36.28±13.84	
①Small cell lung cancers	4	31.20±14.52	<0.05**	23.82±7.61	<0.05**
②Squamous cell carcinomas	18	46.09±9.79	>0.05△	40.37±11.47	>0.05△
③Adenocarcinomas	18	40.77±11.61		32.15±13.11	
With LN metastasis	22	41.01±11.65	>0.05	34.46±15.36	>0.05
Without LN metastasis	18	43.67±11.94		35.69±10.07	
Squamous cell carcinoma					
Poorly differentiated	10	46.40±10.45	>0.05	35.61±11.13	<0.01
Moderate-high differentiated	8	45.48±9.21		49.88±7.17	

LN: lymph node; ① ② ③: group number; *: two-two comparison among groups; **: ① vs ② comparison; △: ① vs ③ or ② vs ③ comparison

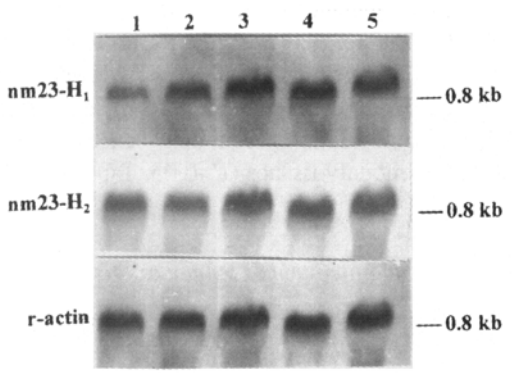

Fig. 1. Northern blot analysis of nm23 gene mRNA in human lung cancer.
1, 3, 5: primary lung cancer tissues; 2, 4: non-cancer pulmonary tissues

The present study examined the mRNA expression of nm23-H_1 and nm23-H_2 in 40 lung cancers by Northern blot hybridization, which showed that nm23-H_2 mRNA expression in poorly differentiated squamous cell carcinoma was significantly decreased from that in moderate-high differentiated squamous cell carcinoma (P<0.01). In addition, nm23-H_1 and nm23-H_2 mRNA expression in small cell lung cancer was also remarkably decreased from that in squamous cell carcinoma. These data suggest that nm23 expression is associated with the histological differentiation of lung cancers. However, the number of patients with small cell lung cancer studied is too small for a final conclusion, more cases are needed for further study. Our data also revealed that there was no significant difference in nm23 mRNA expression between tumor stages, as well as between lung cancers with and without lymph node metastasis in studied cases, which is not in agreement with its antimetastatic role. Dr. Lei and colleagues[6] reported that expression of NDPK/nm23 was correlated with lymph node metastasis of squamous cell lung cancer, but there was no such a correlation in adenocarcinoma. This result is inconsistent with our data, the reason is possibly that the two studies reflected distinct levels of nm23 gene. Moreover, two isotypes of nm23 gene were analyzed separately in our study.

Considering that nm23 gene does not play uniformly a role of metastasis suppression in various kind of tumors, it is proposed that metastasis suppression by nm23 is a tissue specific phenomenon, but its mechanism is still not well demonstrated.[7] Steeg suggested that nm23 might play different functions depending on whether they are normal or defective and on which signal transduction pathway is involved.[8] Okada studied the promoter regions of nm23-H_1 and nm23-H_2 genes, and showed that their promoter regions contained binding sites for known transcriptional factors, but the binding sites of the two genes were not the same. These studies suggested that the two isotypes of the nm23 gene might be regulated independently, and in a cell type specific manner.[9] However, the functioning mechanism of nm23 gene, especially the detailed biochemical pathway it involved,

is currently ill defined; further experiments remain to be done.

Acknowledgements

The authors would like to thank Professor Patricia S Steeg of National Cancer Institute of USA for kindly providing Pnm23-H_1 and Pnm23-H_2 cDNA recombinant plasmids.

REFERENCES

[1]. Steeg PS, Bevilacqua G, Kopper L, et al. Evidence for a novel gene associated with low tumor metastatic potential. J Natl Cancer Inst 1988; 80:200.

[2]. Stahl JA, Leone A, Rosengard AM, et al. Identification of a second human nm23 gene, nm23-H_2. Cancer Res 1991; 51:445.

[3]. Gilles AM, Presecan E, Vonica A, et al. Nucleoside diphosphate kinase from human erythrocytes. J Biol Chem 1991; 266:8784.

[4]. Liu LX, Shi YK, Zhou QH. Research advances on cancer metastasis suppression gene: nm23. Chin J Clin Thorac Cardiovasc Surg 1995; 2:48.

[5]. Leone A, Flatow V, King CR, et al. Reduced tumor incidence, metastatic potential, and cytokine responsiveness of nm23-transfected melanoma cells. Cell 1991; 65:25.

[6]. Lei WD, Zhang RG, Yan SZ, et al. NDPK/nm23 expression and its correlation with lymph node metastasis in human lung cancer. Chin J Oncol 1994; 16:277.

[7]. Haut M, Steeg PS, Willson JK, et al. Induction of nm23 gene expression in human colonic neoplasms and equal expression potential. J Natl Cancer Inst 1991; 83:712.

[8]. Liotta LA, Steeg PS. Clues to the function of nm23 and Awd proteins in development, signal transduction and tumor metastasis provided by studies of dictyostelium discoideum. J Natl Cancer Inst 1990; 82:1170.

[9]. Okada K, Urano T, Baba H, et al. Independent and differential expression of two isotypes of human nm23: analysis of the promoter regions of the nm23-H_1 and H_2 genes. Oncogene 1996; 13:1937.