Chinese Journal of Cancer Research 8(3):222–226,1996.

THE HIGH-LEVEL EXPRESSION OF nm23(NDP) GENE IN NPC

Zheng Tianrong 郑天荣　　Xie Zuofu 谢佐福　　Lin Xiandong 林贤东
Zhang Jingshi 张竟时　　Lu Lili 陆莉莉

Fujian Tumor Hospital, Fuzhou 350014

We have studied the expression of nm23(NDP) in 50 cases nasopharyngeal biopsies with anti-nm23(NDP) antibodies. As a result, the NDP positive rate in nasopharyngeal carcinoma (NPC) (95.54%) markedly increased ($P<0.05$), as compared with that in the normal nasopharyngeal epithelia (50.00–60.00%) and lymphocytes (52.00%). There were cytoplasmic type, nucleus type and mixed cytoplasmonucleus type according to NDP location in a cell. Their positive rates were 64.44%, 15.56% and 20.00% respectively in nasopharyngeal carcinoma. The expression of NDP had no relation with cervical lymphometastases in NPC, and the NDP positive rates had no significance between bilateral cervical lymphometastases and unilateral ($P<0.05$). But the NDP expression had most relation with the NPC staging. The expression rate and the intensity in III or IV stage patients were markedly higher than that in II stage. It points out that the high-level expression of NDP had relation with the rapid cellular proliferation in NPC, and it may indicate the bad prognoses.

Key words: Expression of nm23 gene, Nasopharyngeal carcinoma, Lymphometastases histoimmunochemistry.

The nm23 gene is one of the tumor metastatic inhibitants found recently. In 1988, Steeg and his colleagues isolated it from colony cells of mouse melonoma with different metastatic potentiality. Its product is NDP kinase. The test system with ddifferent metastatic potentiality includes mouse melanoma, N-nitrosourea inducing breast tumor,[1] mouse virus inducing breast tumor[2] and so on; their expressions of nm23 gene are significantly difference. The metastatic potentiality is larger, the expression of nm23 is lower; the converse is also true. The similarly results have found in human breast cancer,[3] liver cancer[4] and lung cancer.[5] But it was reported that the expression of nm23 gene has no relation with the metastasis of human colon tumor,[6] late thyroid carcinoma[7] and so on, whereas it may have relation with the tumor cellular proliferation. The lymphometastases are early in NPC, it is said that the metastatic rate is high.[8] However, up to date it is unknown how nm23 genes express in NPC, and that the relation exists in lymphometastasis of NPC. Now we study the expression of nm23 gene in human NPC using S–P histoimmunochemical techniques.

Accepted May 4, 1996

MATERIALS AND METHODS

Materials

All 50 nasopharyngeal biopsies were collected from our radiotherapeutic department from June to August 1994; male and female were 39 and 11 respectively, and the rate was 3.55:1. The age ranges from 20 to 60 years, and the mean age was 42. The pathologic diagnoses showed 45 cases with low differentiated squamous carcinoma, one case with vacuolate nucleoid carcinoma, one case with low

differentiated adenoma, and three cases with chronic nasopharyngeal inflammation. According to international P-TNM staging criteria, there were no I stage case, 8 II stage cases, 25 III stage cases and 14 IV stage cases.

Ready to use nm23(NDP) polycolony antibodies (made by Oncor Ltd. USA) were all bought from Fuzhou Manxing Biologic Technical Developing Corporation.

Method

The specimens were located with 10% Formalin, wrapped with paraffin wax, and made every slice in 5 μm. The histoimmunochemical techniques adopted S–P techniques, indicated in consultative documents.[9] According to histoimmunochemical dye, it was positive that the cytoplasm and/or nuleus were dyed to brown and it was markedly higher than the background color. It was counted for 200 cells respectively in four sight-field of microscope. According to the percentage of positive cells, there were three degrees: +, the positive cells was under 30%; ++, it ranges from 30% to 70%; +++, it was over 70%.

Statistic Dealing

The differences of all data were judged with x^2 test.

RESULTS

The Expression of nm23(NDP) Gene Existed Both in NPC Tissue and in Non-cancer Tissue

The results are outlined in Table 1 and Figure 1, 2. The total positive rate of nm23(NDP) in NPC tissue was higher by 66.6% to 74.6% than that in normal morphological stratified squamous epithelium, psudostratified columnar epithelium and lymphocytes ($P<0.01$). The high expression rate (+++) of nm23 (NDP) in the cancer tissue increased significantly, as compared with that in the normal squamous epithelium, psudostratified columnar epithelium and lymphocyte ($P<0.01$). Among B cell lymphocytes, whereas, the whole lymph vacuole was positive in one case. NDP was mainly located in cytoplasm in 64.44% cases; located in nucleus in 15.56%; located both in cytoplasm and in nucleus in 20.00%.

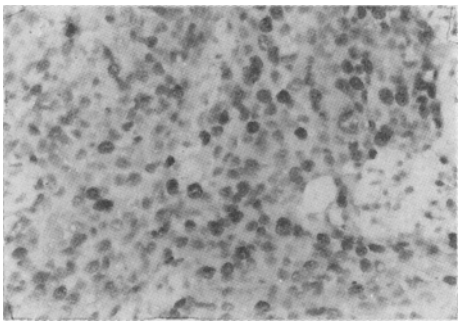

Fig. 1. The nm23(NDP) in the tumor tissue of NPC and it locates in the nucleus. S–P histoimmunochemical dye, × 40.

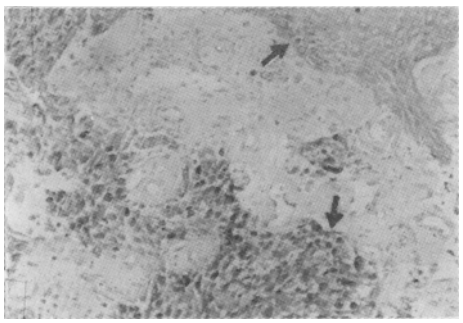

Fig. 2. The nm23(NDP) express both in the tumor tissue and in the normal squamous epithelium of NPC, and it locates in the cytoplasm (↑). S–P histoimmunochemical dye, × 10.

The Relation between the Expression of nm23(NDP) and Histologic Classification in NPC

33 cases had NDP expression among 35 cases of low differentiated squamous carcinoma; one case of vacuolar nucleus carcinoma and one case of low differentiated adenoma also did. The amount of the later two cases was too small to test it significantly.

The Relation between the Expression of nm23(NDP) and Clinic Staging of NPC

As shown in Table 2, the total expression rate in III and IV stage increased significantly as compared with that in II stage ($P<0.05$). The NDP high expression (+++) positive rates had no significance

between II stage and IV stage ($P>0.05$), but it increased significantly in III and IV stage as compared with II stage ($P<0.05$).

The Relation between the Expression of nm23(NDP) and Cervical Lymphometastases

It is outlined in Table 3. The NDP total positive rate had no significance between bilateral and unilateral cervical lymphometastases ($P>0.05$). The lymphometastases rate was 97.73% (43:44) in the NDP positive cases; and it was 50.00% (1:2) in the NDP negative cases.

Table 1. The expression of nm23(NDP) in human nasopharyngeal biopsies

Tissue type	Cases	NDP				TPR (%)	*P*
		+	++	+++	−		
Cancer tissue	47	0	9 (19.15)	36 (76.60)	2	95.74	<0.005*
Squamous epithelium	30	11 (36.67)	4 (13.33)	3 (10.00)	12	60.00	
Columnar epithelium	10	3 (30.00)	1 (10.00)	1 (10.00)	5	50.00	>0.05
Lymphocyte	50	20 (40.00)	5 (10.00)	1 (2.00)	24	52.00	
P		>0.05	>0.05	<0.005			

The number in parentheses are the percentage which the NDP immuno-dyed intensive cases covers the total cases including different tissue types. TPR means total positive rate *P results from comparing the TPR of tumor tissue with the TPRs of normal tissues

Table 2. The relation between the expression nm23(NDP) and the NPC staging

Staging	Cases	NDP				TPR (%)	*P*
		+	++	+++	−		
II	8	0	3 (37.50)	3 (37.50)	2	67.50	
III	25	0	4 (16.00)	21 (84.00)	0	100.00	<0.05
IV	14	0	2 (14.29)	12 (85.71)	0	100.00	<0.05
P			<0.05	<0.05			

The number in parentheses are the percentage which the NDP immuno-dyed intensive cases covers the total cases including different tissues types. TPR means total positive rate. All the P result from comparing II stage and III stage with IV stage.

Table 3. The relation between the expression of nm23(NDP) and the cervical lymphometastases in NPC

Types of the CLM	Cases	NDP			Positive rates (%)
		++	+++		
Bilateral	13	2 (15.38)	10 (72.92)	1	92.31
Unilateral	32	4 (12.50)	27 (84.38)	1	96.88
Without	2	1 (50.00)	0	1	50.00
P		>0.05	>0.05		>0.05

The P results from comparing the bilateral.CLM with unilateral CLM, only 2 without CLM does not compare with the CLM types. CLM means cervical lymphometastases.

The Expression of nm23(NDP) Related to Distant Metastases of NPC

Among 45 cases with NDP positive NPC, one patient had bone metastases, and the distant metastatic rate was 2.22%. There was no distant metastases between 2 cases with NDP negative NPC.

DISCUSSION

Mitchell H. and his colleagues reported nm23 gene expressed not only both in human colon tumor tissue and normal colon mucous membrane, but the expression of nm23 gene increased markedly in the former as compared with the later in the same amount of cases.[8] Our results shows that nm23 gene existed in human NPC tumor tissue was significantly higher than that in normal nasopharyngeal tissue or lymphocytes ($P<0.05$). It indicated that nm23 gene affected the normal tissue growth. It is still unknown that the high expression of nm23(NDP) in NPC was the result or the cause of carcinoma formation.

Many data stated that the decrease of cancer metastatic abilities had relation with the increase of nm23 expression.[1~5] But some researchers reported that the relation did not exist in colon carcinoma and thyroid carcinoma.[6] M. Zou and his colleagues stated that the high-level expression of nm23 may relates with the rapid proliferation of cancer cells in thyroid carcinoma, and it had no relation with the carcinoma metastases.[7] We had the same results with M. Zou. The total positive rate and high expression positive rate of nm23 gene had no significance between bilateral and unilateral cervical lymphometastases ($P>0.05$), whereas, the cervical hymphometastatic rate was higher in the positives than that in the negatives of nm23 gene expression. The cervical lymphometastatic cases was too small to significantly testing, but it at least indicated that the high expression of nm23 gene had no relation with the potential nasopharyngeal lymphometastases.

The expressive rate and the intensity of nm23 gene in III and IV stage NPC were higher than that in II stage NPC ($P<0.05$). It indicated that the high expression related with the bad prognoses of NPC patients.

Recently it has been proved that NDP located in the cytoplasm by Toshihiro N and his colleagues using anti-NDP polycolony antibodies, and by Y Tokunaga and his colleagues using anti-NDP monocolony antibodies.[10] But our results show that NDP locating in the cytoplasm covers only 64.44% cases; NDP locating in the nucleus covered 15.56% NDP, locating both in the cytoplasm and in the nucleus at the same time covered 20.00%. NDP is important to cellular energy metabolism, glucogen synthesis and the metabolism of nucleotide,[10] therefore, the difference indicated that the expression types of NDP are different according to different types of cells or during different periods of the cellular cycle, and that NDP have different functions on the metabolisms.

REFERENCES

1. Steeg PS, Bevilocqua G, Kopper L, et al. Evidence for a novel gene associated with low metastic potential. J Natal Cancer Inst 1988; 80:200.
2. Steeg PS, Bevilacqua G, Rosengard M, et al. Altered gene expression in tumor metastasis: The nm23 gene. In: Schirrmacher V, Schworrtz R, eds. Cancer Metastasis. Berlin: Springer-Verlag. 1989; 48.
3. Bevilacqua G, Sobel ME, Liotta LA, et al. Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas with lymph node involvement and their histopathological indicators of high metastatic potential. Cancer Res 1989; 49: 5185.
4. Toshihiro N, Akira O, Kazuhiko N, et al. Expression in human hepatocellular carcinoma of nuclelside diphosphate kinase, a homologue of the nm23 gene product. J Natal Cancer Inst 1992; 84(17):1349.
5. 宙文东、张汝刚、闫水忠、等. nm23 基因在人肺癌中的表达及与肺癌淋巴结转移的关系. 中华肿瘤杂志 1994; 16(4):277.
6. Nitchell H, Patricias S, James KV, et al. Induction of nm23 gene expression in human colonic neoplasms and equal expression in colon tumors of high and low metastatic potential. J Natal Cancer Inst 1991; 83 (10):712.
7. Zou M, Shi Y, al Sedairy S, et al. High levels of nm23 gene expression in advanced stage of thyroid carcinoma. Br J Cancer 1993; 68:385.
8. 汤钊猷, 主编. 现代肿瘤学. 上海: 上海医科大学出版社. 1993; 588.
9. Shi ZR, Itzkowitz SH, Kim YS. A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcinoma tissues. J

Histochem Cytochem 1988; 96(3):317.
10. Yuji T, Takeshi U, Koechi F, et al. Reduced expression of nm23-H1 but not of nm23-H2, is concordant with the frequency of lymph node metastasis of human breast cancer. Int J Cancer 1993; 55:66.

A CASE OF OLFACTORY NEUROBLASTOMA

Cui Fengjiu 崔凤九 Jin Kuilong* 金奎龙 Jin Yingjin* 金英锦 Zhu Yongzhe* 朱永哲

*Department of Oncology, *Department of Electron-microscopy, Affiliated Hospital, Yanbian Medical College, Yanji 133000*

Patient

Male, 52 of age. For 8 months he suffered from nasal obstruction, discharge, repeated nosebleed and for one month also from headache. He came to the clinic on November 9, 1993. 11 months before he had an operation of nasal polyp.

Examination

Swelling on the left chin, projection of the left eye, blocking of neoformation in the left nasal cavity, erosion of surface, no active bleeding and the nasal septum moved to the right.

Observation with CT

Lumps of soft tissue accumulated in the sinuses of upper jaw projected into the nasal cavity and the bone of side wall of the sinuses was destroyed and the nasal septum was excavated to the right under the oppression and ethmoid sinus was full of shades of soft tissue and the bone of the nasal septum was absorbed.

Pathological Examination

Under the microscope the tumor cells were round or oval and they were separated into the balls of different sizes by connective tissues, and there were few cytosols. The nuclei were oval or round or in the form of chrisanthemin. Under the CT, nuclei were oval and the cells were arranged into balls of the form of chrisanthemin and there were neurosecretory granules and formation of synapses and on the section of the nerve process there were small vesides of synapses.

Pathological Diagnosis

Olfactory neuroblastoma in the left nasal cavity. On November 15 the radiation therapy was performed (D_T 8Gy/9f) and after that the patient came no longer.

Discussion

Some report on this kind of disease said that somebody found olfactory neuroblastoma while examining the cells of nasal discharge. At present, the nerve fiber was confirmed with Bodium staining and eosinophilic substance in chrisanthemin-formed balls with Mucicarmin staining and enzyme antibody method is important basis in the diagnosis of this disease. The diagnosis was more certain when neurosecretory granules and synapses of nerve cells were seen through CT.

(Accepted August 10, 1996)