Chinese Journal of Cancer Research 10(1): 49–53, 1998.

Clinical Observations

EXPRESSION OF NM23-H1 GENE PRODUCT IN NASOPHARYNGEAL CARCINOMA AND ITS CLINICAL SIGNIFICANCE

Guo Xiang 郭翔　Min Huaqing 闵华庆　Shao Jianyong 邵建永
Hou Jinghui 侯景辉

Department of NPC, Tumor Hospital, Sun Yat-sen University of Medical Sciences, Guangzhou 510060

Objective: The nm23 gene is one of the tumor metastatic suppressor genes. The expression of nm23-H1 has been reported to be inversely associated with metastatic potentiality in a number of human carcinomas, including breast, colorectal, gastric, hepatocellular and gallbladder carcinomas. In this study, the immunohistochemical staining of nm23-H1 protein in human nasopharyngeal carcinoma (NPC) was examined, and the relationship between nm23-H1 and both metastasis and prognosis of patients with NPC was also investigated. Methods: Routine LSAB immunohistochemistry with the nm23-H1 monoclonal murine antibody was employed to study the expression of nm23-H1 protein in 95 paraffin-embedded specimens of NPC treated at our hospital. The clinical pathologic data and results of follow-up were also retrieved. Comparisons between patients with and without expression of nm23-H1 protein with respect to metastasis, loco-regional recurrence and survival were performed using Log rank test. Multivariate prognostic analyses were performed by using Cox's regression model. Results: Nm23-H1 negative expressive tumors were associated with a higher incidence of lymph-node metastasis (86.7%) than those of nm23-H1 positive (48.6%, $P<0.01$). Nm23-H1 negative expressive tumors were associated with a high incidence of recurrence and distant metastasis after radiotherapy ($P<0.05$). A significant association was found between expression of nm23-H1 and prognosis ($P<0.01$). The expression of nm23-H1 indicated favorable prognosis. Conclusion: It was suggested that nm23-H1 negative expression was significantly associated with lymph-node metastasis, recurrence and distant metastasis. Nm23-H1 may have value for predicting the prognosis of NPC.

Key words: nm23-H1, Tumor metastatic suppressor gene, Nasopharyngeal carcinoma, Prognosis.

Accepted August 5, 1997

The nm23 gene was originally identified by Steeg et al.[1] in NCI by screening of cDNA libraries with differential hybridization between two murine melanoma sublines from K-1735 melanoma cell line, which have low and high metastatic potential respectively. It is suggested that nm23 may function suppresser gene for tumor metastasis by the fact that the expression level of nm23 gene from less metastatic murine melanoma subline was 10 times higher than that of highly metastatic cell line. Thereafter, an inverse relationship between metastatic potential and expression level of nm23 gene was reported in many metastatic model systems and some human malignancies (breast carcinoma, carcinoma of large intestine, gastric carcinoma and hepatocellular carcinoma),[2–5] although several studies show that there is no relationship between the expression of nm23 gene and metastasis.[6,7] Isotype specific studies on breast neo-

plasms have indicated that it is nm23-H1, but not nm23-H2, that correlates with metastasis.[8]

In this study, we analyzed the immunohistochemical staining characteristics of nm23-H1 protein in 95 cases of NPC using monoclonal antibodies, and thereby investigated the relationship between protein immunoreactivity and prognosis in NPC.

MATERIALS AND METHODS

Tissue Samples

Tumor specimens of 95 cases were paraffin embedded archival tissues of NPC from initial biopsies in Tumor Hospital, Sun Yat-sen University of Medical Sciences, from November 1985 to December 1987. These tissues were from 75 males and 20 females, aged between 16 and 66 years. Classification of tumor stage was defined according to the "92's NPC staging" system, which was recommended to be used in China by Chinese Society for Radiation Oncology at the National Conference for Radiation Oncology in November 1992.[9]

Immunohistochemistry

4 μm paraffin sections of tissue were prepared, dewaxed in xylene, and then treated with microwave heating at 92℃ for 10 min in citrate buffer (10 mmol/L, pH 6.0) for antigen retrieval. After blocking of endogenous peroxidase, routine labelling streptavidin biotin (LSAB) immunostaining with diaminobenzidine was applied to sections incubated overnight with a monoclonal antibody to the nm23-H1 protein (NM 301: Santa Cruz Biotechnology, Inc. USA: LSAB Box: DAKO Ltd. Japan) at 1:100 dilution. The sections were counterstained with hematoxylin, cleaned, and mounted.

Assessment

Sections were assessed by two independent qualified pathologists, ignorant of the clinical details. According to the stained products of nm23-H1 located in the cytoplasm of tumor cells as yellowbrown granules, a two-point scale was used in the assessment as follows: negative (−): no significant tumor cell staining; positive (+): cytoplasmic staining of tumor cell.

Follow-up

Follow-up of all patients was until the time of death or over 5 years. The median follow-up was for 54 months with the mean being 49.4 months, range 9–98 months.

Statistical Analysis

All analyses were carried out using SPSS 6.0 software package. Tumor and patient variables were compared employing the chi-square test. Survival between two groups of patients was compared using the log rank test. Cox's proportional hazards regression model was used to analyze the independent prognostic effect of each variable.

RESULTS

Expression of nm23-H1

Staining for the products of nm23-H1 gene was mostly cytoplasmic, but nuclear staining was also seen in some cases (Figure 1). The rate of positive cytoplasmic staining in this study was 36.8% (35/95). The relationships between nm23 expression and clinical outcomes were shown in Table 1. All *P* values were two-tailed. There was a highly significant association of nm23-H1 negative staining with regional nodal metastasis in NPC. The negative staining of nm23-H1 was also significantly associated with the local or regional recurrence and distant metastasis after radiotherapy.

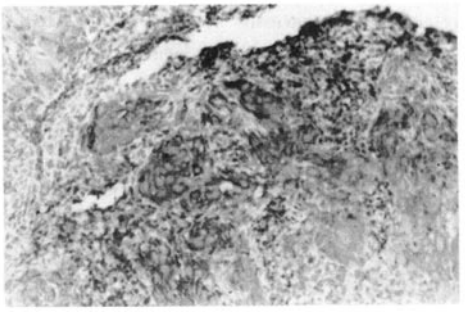

Fig. 1. nm23-H1 protein expression in NPC. Positive cells showed diffuse cytoplasmic staining. (LSAB method, counterstained with hematoxylin; × 160)

Table 1. The relationship of nm23-H1 expression and both clinicopathologic findings and clinical outcomes

Clinical data	nm23-H1		x^2	P values
	−	+		
Sex				
Male	50	25		
Female	10	10	1.8849	>0.05
Regional LN				
N0	8	18		
N1	17	6		
N2	31	10	16.2065	<0.01
N3	4	1		
Primary tumor				
T1	3	2		
T2	25	11		
T3	23	14	1.4112	>0.05
T4	9	8		
Clinical staging				
I, II	13	8		
III, IV	47	27	0.0182	>0.05
Loco-regional recurrence				
No	40	30		
Yes	20	5	4.1361	<0.05
Distant metastasis				
No	42	31		
Yes	18	4	4.2844	<0.05

The Survival Curves of the Two Groups

The overall patients were divided into two groups according to with or without expression of nm23-H1 protein. The survival curves of them were demonstrated in Figure 2 and significant difference was shown after the log rank test (x^2=7.23, P<0.01). This result showed that nm23-H1 protein expression had a significant correlation with prognosis of NPC; a higher survival rate was demonstrated in expression group. Also noted was that the expression of nm23-H1 was an important prognostic indicator in patients with NPC while the data were analyzed by Cox's proportional hazards regression model (Table 2).

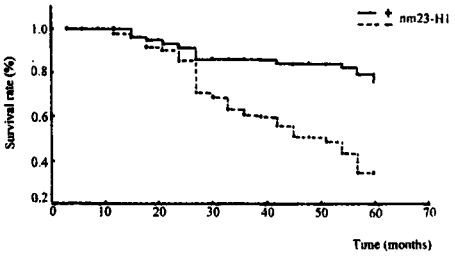

Fig. 2. Survival curves of patients with NPC, subdivided according to nm23-H1 protein expression (P<0.01, by Log Rank test).

Table 2. The analysis of 95 cases of NPC by Cox' s proportional hazards regression model

Factors	B	S.E.	P
Age	- 0.0092	0.0106	0.3816
Sex	0.2012	0.2703	0.4566
Lymph node	- 0.2160	0.1366	0.1140
Staging	0.1421	0.1767	0.4212
Recurrence	1.4336	0.3045	0.0000
Metastasis	1.5182	0.2973	0.0000
nm23-H1	- 0.5846	0.2561	0.0224

DISCUSSION

Distant metastasis is a main cause of death in many malignancies. The procedures of tumor development and tumor metastasis are two independent biological processes controlled by some correlated genes, but have somewhat relationships. The metastatic behavior of malignancies involves a series of genetic events with abnormal activation of metastasis-related genes or inactivation of metastasis suppressor genes.[10] Nm23 gene is known as a best representative of metastasis suppressor genes, which was originally cloned and defined from two K1735 murine melanoma sublines with different metastatic potentialities. It is reported that the expression level of the nm23-H1 mRNA is inversely associated with distant metastasis and poor prognosis in several type of human malignancies, i.e. breast cancer, gastric cancer, large intestine cancer and hepatocellular carcinoma; and deletion of nm23 gene is significantly associated with tumor metastasis.[2–5] There are at least 2 isotypes of human nm23 genes, namely nm23-H1 and nm23-H2, which are 88 per cent homologous to each other.[11] The nm23-H1 gene, which is a series of 533 nucleotides, maps to the chromosome 17q22 and encodes a 17 KD polypeptide consisted of 152 amino acids.[11] A remarkably high degree of amino acid sequence homology is found between nm23 protein encoded by nm23 gene and nucleoside diphosphate kinase (NDPK). The products of nm23 gene are intracytoplasmic and intranuclear localization. Leone et al.[12] discovered that metastatic potentiality could be transfected to human breast cancer cell line by nm23-H1cDNA, but not by nm23-H2cDNA. This suggests that nm23-H1 gene has more important role in cancer metastasis. Tokunaga et al.[8] reported that expression of nm23-H1 gene rather than nm23-H2 gene appeared more closely related to metastasis in human breast cancer.

In present study, immunohistochemical detection of nm23-H1 protein was employed in 95 NPC. There was a significant correlation between negative expression of nm23-H1 and lymph node metastasis. The rate of lymph node metastasis in nm23-H1-expression-negative group was 86.7%, while it was only 48.6% in nm23-H1-expression-positive group; there was significant difference between two groups ($P<0.01$). In follow-up of the whole patients, it was found that in nm23-H1-expression-negative group, 33% occurred locoregional relapse after radiotherapy, 33% occurred distant metastasis, while it were 14.8% and 11.4% respectively in nm23-H1-expression-positive group ($P<0.05$). Our results were consistent with the researches in hepatocellular carcinoma, gastric carcinoma, breast carcinoma and malignant melanoma. The inverse correlation of reduced nm23 expression to the metastasis and relapse of malignant melanoma was reported by Florenes et al.[13] in the research of the level of nm23-H1 mRNA in 33 malignant melanomas. Sheng et al.[14] reported that the low level expression of nm23 gene associated with infiltration and lymph node metastasis in gastric carcinoma. Significant correlation of the expression level of nm23-H1 mRNA to the lymph node metastasis and tumor cell differentiation in primary breast carcinoma was reported by Hennessy et al.[2], while they found that all of the patients with reduced expression of nm23-H1 mRNA relapsed 3 years later after operation. Yamaguchi et al.[5] observed an inverse relationship between nm23-H1 expression and intrahepatic metastases of hepatocellular carcinomas; the patients with reduced expression of nm23-H1 had shorter survival period. In present study, no relationship was found between nm23-H1 expression and sex or tumor grade.

The function of nm23 gene product in relation to regional lymph node metastasis and distant metastasis of NPC remains undetermined. A notably high degree of amino acid sequence homology is found between nm23 protein and nucleoside diphosphate kinase (NDPK), and it was also reported that nm23 protein and NDPK shared similar biochemical characteristics. NDPK has many biological functions relating to nucleoside-triphosphate-dependent cellular activities. It may also take part in signal transduction of G-protein, participate regulation of microtubule polymerization in the mitotic spindle, and participate DNA synthesis. So it is suggested that NDPK may influence the cellular differentiation and activation of oncogenes along with alternating the cellular skeleton and reaction to cyclins resulting in cellular movements. This may partially explain the phenomenon of tumor cell infiltration and metastasis. But Macdonald et al.[15] reported that the activity of NDPK had no relationship with metastatic suppression of K-1735 murine melanoma cell line; instead of it, the phos-phorylation level of serine 44 of NDPK was asso-ciated with metastatic suppression.

Another important discovery in this study was

that the expression of nm23-H1 protein was correlated with the prognosis of NPC who were nm23-H1-expression-positive had better 5-year survival rate after radiotherapy (Figure 2). This implicated that nm23-H1 product may be a prognostic indicator in NPC. This result is consistent with other researches in gastric carcinoma, malignant melanoma, breast carcinoma and colorectal carcinoma. It was suggested that pathological diagnosis along with detection of nm23-H1 product in tumor tissue should be valuable in new case of NPC in the evaluation of the prognosis of individual patient, so as to draw up more practical individual treatment modality. Intensive follow-up should be emphasized in the patients of nm23-H1-expression-negative NPC.

In the present study, although nm23-H1 gene product was significantly associated with the prognosis and lymph node metastasis in NPC, the biological function of nm23-H1 protein was still unclear.

REFERENCES

1. Steeg PA, Bevilacqua G, Kopper L, et al. Evidence for a novel gene associated with a low tumor metastatic potential. J Natl Cancer Inst 1988; 80:204.
2. Hennessy C, Henry JA, May FEB, et al. Expression of the anti-metastatic gene nm23 in human breast cancer: an association with good prognosis. J Natl Cancer Inst 1991; 83:281.
3. 杨春康, 许东波, 林永坤. nm23 基因产物在大肠癌中的表达及其临床意义. 癌症 1996; 15(4):262.
4. Kodera Y, Isobe K, Yamauchi M, et al. Expression of nm23-H1 RNA levels in human gastric cancer tissues: a negative correlation with nodal metastasis. Cancer 1994; 73(2):259.
5. Yamaguchi A, Urano T, Goi T, et al. Expression of human nm23-H1 and nm23-H2 protein in hepatocellular carcinoma. Cancer 1994; 73(9): 2280.
6. 李家良, 李甘地, 杨光华. NDPK/nm23 基因在人纤维肉瘤中的表达及其与转移的关系. 中华病理杂志 1996; 25(3):174.
7. Ro YS, Jeong SJ. Expression of the nucleoside diphosphate kinase in human skin cancers: an immunohistochemical study. J Kerean Med Sci 1995; 10(2):97.
8. Watanabe J, Sato Y, Kuramoto H. Expression of nm23-H1 and nm23-H2 protein in endometrial carcinoma. Br J Cancer 1995; 72(6):1469.
9. 吴晓华, 沈忠英, 汤雪明. 肿瘤转移抑制基因 nm23 研究新展. 生命科学 1996; 8(3):18.
10. Varasco L, Caligo MA, Simi P, et al. The nm23 gene maps to human chromosome band 17q22 and shows a restriction fragment length polymorphism with Bg/II. Genes Chrom Cancer 1992; 4:84.
11. 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.
12. Tokunaga Y, Uranc T, Furukawa K, et al. Reduced expression of nm23-H1 but not nm23-H2 is concordant with the frequency of lymph node metastasis of human breast cancer. Int J Cancer 1993; 55:66.
13. Florenes VA, Aamdal S, Myklebost O, et al. Levels of nm23 messenger RNA in metastatic malignant melanomas: inverse correlation to disease progression. Cancer Res 1992; 52:6088.
14. 张声, 叶圣华, 林华. nm23 与 P21 在胃癌中表达的意义. 癌症 1996; 15(1):26.
15. Macdonald NJ, Rosa ADL, Bendedict MA, et al. A serine phosphorylation of nm23, and not its nucleoside diphosphate kinase activity, correlates with suppression of tumor metastasis potential. J Biol Chem 1993; 268 (34):25780.