

# nm23 expression in gastric carcinoma and its relationship with lymphoproliferation

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**Subject headings** stomach neoplasms; nm23 protein; gene expression; lymphoproliferation; lymphocytes

## INTRODUCTION

Tumor spread is a complex biological process closely related to tumor growth, which is regulated by many genes within the cell. Recent studies have revealed that nm23 is intimately related to tumor metastasis in its biochemical nature, structure and function and its regulating role of the gene itself<sup>[1]</sup>. In this study, gene product nm23 expression was performed in 97 cases of gastric cancer and observations were made on its relationship to hyperplasia of lymphatic tissue.

## MATERIALS AND METHOD

Specimens were collected from 97 cases of gastric cancer treated by radical surgery together with 482 enlarged regional lymph nodes (including 214 with reactive hyperplasia and 268 showing cancer metastases). Specimens were fixed in 100mL/L-formalin solution and embedded in paraffin wax. Sections of 4μm in thickness were made and routinely stained with HE stain. In accordance with the literature<sup>[2]</sup>, gastric carcinoma was divided into: stage T1, where cancer tissue invades the mucosa or submucosa; stage T2, with invasion of muscular layer; stage T3, with invasion of serosa; stage T4, with invasion of tissue outside the serosa or of adjacent organs.

## Observation of lymphocytes surrounding the cancer

Lymphocytes in the advancing aspect of cancerous invasion were observed but excluding lymphocytes in between cancer nests and the submucosal lymphocytic reaction.

Observations were made separately for each type and each stage of gastric carcinoma.

## Observation of lymph node metastasis

Changes were observed in lymph node metastasis, which were into 4 stages<sup>[3]</sup>: Stage 1, structure of lymph nodes is undamaged. Peripheral sinuses or elsewhere show invasion by solitary or multiple cancer cells which may be scattered or form cancerous foci comprised of 3 - 5 cells each; Stage 2, metastatic cancer cells comprise < 1/3 of surface area of section of lymph gland and usually with intact lymph follicles, dilated lymph sinuses filled with cancer cells and an intact lymph node capsule; stage 3, metastatic cancer cells comprise > 2/3 of cross sectional area with intact lymph node capsule; and stage 4, the lymph node and its capsule are both invaded by metastatic cancer cells, or there is invasion of surrounding fibrofatty tissue, muscle fibres, glands etc. with little residual lymphatic tissue.

## Antibody and staining methods

One section was randomly selected from the sections made from the 4 pieces of tissue obtained from around the cancer and tested for expression of nm23 gene product using the streptomyces antibiotin peroxidase linkage method (S-P). DAB was used coloration, and haematoxylin for background staining.

## RESULT

Expression of nm23 of gastric carcinoma and results of examination for lymphocytes around the cancer are shown in Table 1.

## Relationship between expression of nm23 and reactive hyperplasia in lymph nodes

In each type of gastric cancer showing enhanced expression of nm23, reactive hyperplasia of regional lymph nodes was active, whereas this was diminished in those cases showing negative or weak expression of nm23. High expressivity of nm23 shows positive correlation with the amount of reactive hyperplasia of lymph nodes in the drainage area of the cancer, and the latter was related somewhat with the histological type of tumor. In papillary adenocarcinoma and tubular adenocarcinoma there was a greater amount of reactive hyperplasia, while the hyperplasia was low in adenocarcinoma, with low grade differentiation, mucinous adenocarcinoma and signet ring cell carcinoma.

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Received 1998-07-07

**Table 1 Results of positive expression of nm23, hyperplasia of lymphatic tissue and metastasis in various types of gastric carcinoma**

Type of cancer	Number of cases	Positive expression of nm23 cases (%)	Enlarged lymph nodes (nodes)	Reactive hyperplasia of lymph nodes (nodes)	Cancer metastasis of lymph nodes (nodes)				Lymphocytes around cancer (cases)			
					I	II	III	IV	-	+	++	+++
Papillary adenocarcinoma	8	7(87.5)	34	26	2	4	2	0	1(12.5)	3(37.5)	3(37.5)	1(12.5)
Tubular adenocarcinoma	17	14(82.4)	105	38	4	28	29	6	0(0.0)	3(17.6)	8(47.1)	6(35.3)
Poorly differentiated adenocarcinoma	37	30(81.1)	163	97	2	16	29	19	4(10.8)	7(45.9)	11(29.7)	5(13.5)
Mucinous adenocarcinoma	23	16(69.6)	89	35	1	14	26	13	3(13.0)	12(52.2)	6(26.1)	2(8.7)
Signet ring cell carcinoma	12	9(75.0)	91	18	2	24	32	15	1(12.5)	6(50.0)	5(41.7)	0(0.0)
Total	97	76(78.4)	482	214	1	86	118	53	9(9.3)	41(42.3)	33(34.0)	14(14.4)

**Table 2 Relationship between stage of gastric cancer and expression of nm23 and hyperplasia of lymphatic tissue**

Group	No. of cases	Positive nm23 expression No. (%)	Lymphocytes around cancer cases (%)	Reactive hyperplasia of lymph nodes (nodes)	Metastatic lymph nodes (nodes)
T 1	6	6(100.0)	6(100.0)	48	0
T 2	15	14(93.3)	14(93.3)	52	4
T 3	27	24(85.2)	25(92.6)	63	19
T 4	49	33(67.3)	43(97.8)	51	245
Total	97	76(78.4)	88(90.7)	214	268

### **Relationship between expression of nm23 and lymph node metastasis**

In all types of gastric cancer when positive expression of nm23 protein was enhanced there was generally no spread to the lymph nodes in the drainage area of the cancer. When expression was negative, there was usually metastasis to regional nodes. There was a negative correlation between high expressivity of nm23 and the number and degree of regional lymph node involvement (Table 2).

### **Relationship between expression of nm23 and histological type and depth of infiltration in gastric carcinoma**

There was some relationship between expression of nm23 protein and the histological type of gastric cancer and the depth of invasion. A high positive rate was seen in papillary adenocarcinoma, tubular adenocarcinoma and poorly differentiated adenocarcinoma as compared with mucinous adenocarcinoma and signet ring cell carcinoma, but the difference was not marked ( $P>0.05$ ). Positive rate of nm23 expression decreased as depth of invasion increased. Stages T1 and T2 show marked difference as compared with Stage T4 ( $P<0.01$ ).

### **DISCUSSION**

The appearance of large numbers of lymphocytes around a cancer is the morphological expression of the body's immunological reaction to the tumor.

Tumors can indirectly inhibit the antineoplastic cellular immunity of the host by means of lymphocytes. The degree of inhibition shows a parallel relationship with the degree of malignancy of the tumor<sup>[4]</sup>. A considerable portion of these lymphocytes are immunoresponsive and having lethal activity on tumor cells. They directly prevent tumor growth by releasing lymphokines or through the lethal action of cytotoxins<sup>[5,6]</sup>. Our results are basically the same with those reported in literature. The degree of lymphocytic infiltration around a cancer is related to the stage of the tumor. The covatation degree in the early stage of adenocarcinoma is more serious, and the lymph nodes with reactive hyperplasia are higher in number than those in the late stage. The degree of lymphatic tissue hyperplasia was not significantly related to the age and sex of the patient.

Gene nm23 is a type identified through the CD-NA archives for low grade metastasing melanoma cell line K-1735 of mice using different hybridization technics. In this gene, the levels of mRNA and the encoded protein are markedly lowered in many experimental tumors of high metastatic phenotype, hence it is considered as a metastasis-inhibiting gene. Human nm23 gene has two subtypes: nm23-H1 and nm23-H2<sup>[7,8]</sup>, located in human chromosome number 17 in its long arm in the vicinity of the centromere, its encoded product being a 17kD protein composed of 152 aminoacids<sup>[9]</sup>. The relationship between human nm23 genetic protein and nu-

cleoside diphosphate kinase (N DP K) expression universally present inside cells and tumor spread and prognosis, is still controversial in the literature<sup>[10,11]</sup>. In this group of 97 cases, positive expression is seen in 78.4% which is intimately related to the degree of lymphatic tissue hyperplasia. Lymphocytic infiltration was found around the cancer in 88 cases accounting for 90.7% of the total. Marked surrounding infiltration was seen with positive expression of nm23 in 79% of 14 such cases, while in 9 cases with absence of lymphocytic infiltration, nm23 expression was found in 32.5%, difference being significant between the two groups ( $P < 0.01$ ).

Lymph nodes in the area of drainage of the cancer were presented with a stage of reactive hyperplasia and a stage of metastasis, each showing corresponding characteristic changes in histological structure, and difference in quantity and in degree<sup>[12]</sup>. In this study, the degree of reactive hyperplasia of the regional lymph nodes and lymph node metastasis is related closely to nm23 gene expression. In this group, of the 482 enlarged lymph nodes, 21 had reactive hyperplasia and 268 had metastasis. When reactive hyperplasia is large in number and severe in degree, the positive expression rate of nm23 is increased, if opposite, the rate decreased. When the number of metastatic lymph nodes is large and the degree of involvement is severe, the positive expression of nm23 is reduced, and increased if opposite. This shows that level of expression of nm23 is intimately related to enlargement of lymph nodes in the drainage area. This means specifically, that expressivity of nm23 is in direct ratio to the amount and degree of reactive hy-

perplasia in lymph nodes, but inverse ratio to the number and degree of lymph node involvement in metastasis. It is likely that nm23 gene inhibits the metastatic action of the tumor after cell malignancy transformation. Such close relationship of nm23 with inhibition of tumor spread and the reduction of lymphatic tissue hyperplasia in gastric carcinoma awaits further investigations. Expression of nm23 gene which helps understand hyperplasia and metastasis of lymphatic tissue, and evaluate the depth of invasion of gastric carcinoma provides a useful method in radiotherapy and chemotherapy.

## REFERENCES

- 1 Hennessy C, Henry JA, May FE, Westley BR, Angus B, Lennard TW. Expression of the antimetastatic gene nm23 in human breast cancer: an association with good prognosis. *J Natl Cancer Inst*, 1991;83:281-285
- 2 Liu TH, Li WH. Diagnostic pathology. 1st ed. Beijing: People's Health Press, 1994:68-74
- 3 Wang YK, Ma NX, Wang XL, Sun MG. Histopathological study on the phase of metastatic carcinoma of lymph node and its significance. *Cancer Res Clin*, 1996; 8:76-78
- 4 Ioannides CG, Whiteside TL. T cell recognition of human tumors: implications for molecular immunotherapy of cancer. *Clin Immunol Immunopathol*, 1993; 66: 91-106
- 5 Alexander D, Shiroo M, Robinson A, Biffen M, Shivan E. The role of CD45 in T-cell activation-resolving the paradoxes. *Immunol Today*, 1992;13:477-481
- 6 Fujii Y, Okumura M, Inada K, Nakahara K. Reversal of CD45R isoform switching in CD8+T cells. *Cell Immunol*, 1992;139:176-184
- 7 Steeg PS, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA. Evidence for a novel gene associated with low tumor metastatic potential. *J Natl Cancer Inst*, 1988;80:200-204
- 8 Stahl JA, Leone A, Rosengard AM, Porter L, King CR, Steeg PS. Identification of a second human nm23 gene, nm23 H2. *Cancer Res*, 1991;51:445-449
- 9 Backer JM, Mendola CE, Kovacs I, Fairhurst JL, O'Hara B, Eddy RL. Chromosomal localization and nucleoside diphosphate kinase activity of human metastasis-suppressor genes nm23-H1 and nm23-H2. *Oncogene*, 1993;8:497-502
- 10 Cohn KH, Wang FS, Desoto-Lapaix F, Solomon WB, Patterson LG, Arnold MR. Association of nm23-H1 allelic deletions with distant metastasis in colorectal carcinoma. *Lancet*, 1991;338:722-724
- 11 Royds JA, Cross SS, Silcocks PB, Scholefield JH, Rees RC, Stephenson TJ. nm23 'anti-metastatic' gene product expression in colorectal carcinoma. *J Pathol*, 1994;172:261-266
- 12 Wang YK, Fu JB, Liu L, Dong RC. Histopathological study of the reaction hyperplasia of lymph node in the cancer drainage area. *Chin J Clin Oncol*, 1993; 20:723-724

Edited by MA Jing-Yun