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Expression of p53 and NDP-K/nm23 in Gastric Carcinomas
— Association with metastasis and clinicopathologic parameters —

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To evaluate the role of p53 and NDP-K / nm23(nm23) protein as a prognostic factor and their relation to metastasis of cancer, we studied metastatic and nonmetastatic gastric carcinoma specimens by immunohistochemical staining. Among the 101 specimens examined, 37(36.6 %) showed positivity in staining for p53 protein and 64(63.4 %) showed no detectable p53 protein in tumor cells. p53 overexpression was correlated with depth of invasion, lymphatic invasion, lymph node metastasis and distant metastasis. Out of 101 specimens, 35 cases had no staining for nm23. 62 cases(61.4 %) exhibited a cytoplasmic staining on most cells and 42 cases(41.6 %) had nuclear staining. In 16 of 101 cases(15.8 %), a mild to moderate membranous staining was observed in some cells. Cytoplasmic nm23 expression was negatively correlated with lymph node metastasis($P<0.01$) and distant metastasis($P<0.01$). The nuclear nm23 expression showed negative correlation with depth of invasion($P<0.01$), lymphatic invasion($P<0.01$), lymph node metastasis($P<0.01$), and distant metastasis($P<0.04$). The membranous nm23 expression revealed negative correlation with lymphatic invasion($P<0.02$), lymph node metastasis($P<0.01$) and distant metastasis($P<0.02$).

Key Words : *p53, nm23, Gastric carcinoma, Metastasis, Prognosis*

INTRODUCTION

p53 is a nuclear phosphoprotein that can regulate cell proliferation and suppress tumor growth(Vogelstein and Kinzler, 1992). Mutation in the p53 gene is turning out to be the most common genetic alteration in various human cancers. Mutations in the p53 gene have been reported in a variety of human tumors, and in selected malignancies overexpression of p53 has been associated with a poor prognosis in breast(Thor et al., 1992) and prostatic carcinomas(Visakorpi et al., 1992). Abnormalities of the p53 gene have been related to dissemination in colorectal carcinomas (Remvikos et al., 1990). A correlation between abnormal p53 expression with tumorigenesis and metastatic potential has also been noted in colorectal cell lines (Trainer et al., 1988).

The nm23 gene was initially isolated by differential colony hybridization of cDNAs derived from low and high metastatic murine K-1735 melanoma cells(Steeg et al., 1988). The nm23 gene is localized on chromosome 17q22(Varesco et al., 1992). The murine nm23 and the two human genes, nm23-H1 and nm23-H2, encode 17 kilodalton proteins that are 90 % identical in predicted amino acid sequence(Steeg et al., 1988). The nm23 codes for a gene product that is almost

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homologous in the amino acid sequence with NDP(nucleoside diphosphate) kinase A in human erythrocytes(Gilles et al., 1991). So we used the monoclonal antibody NDPK-A/nm23, which has specificity for human NDP kinase A. Expression of nm23 protein has been investigated in a number of tumors and in several has been shown to indicate a less aggressive phenotype(Sawan et al., 1994). For example, in breast cancer, patients with tumors expressing nm23 have a much better outlook. Gastric carcinoma is the most common malignancy in Korea comprising 22.7 % of the total malignancies. It is the most common malignant tumor in male and the second most common malignancy in female(Ministry of Health and Welfare, Republic of Korea, 1993). It is a particularly aggressive cancer, and the 5-year survival rate is only 11 %(Cancer Research Campaign 1990). In this study, we investigate the p53 and nm23 expression in early, advanced and metastatic gastric carcinomas and their relation to clinicopathological prognostic parameters and metastasis.

MATERIALS AND METHODS

Materials

One hundred and one patients with gastric carcinomas were studied. The 75 gastrectomized specimen were obtained from the patients who underwent gastrectomy between March 1991 and February 1995 at Our Lady of Mercy Hospital. For the clinical staging all patients were evaluated by fiberoptic endoscopy, plain radiography, upper gastrointestinal series, computed tomography and bone scan. The twenty-six gastric biopsy specimens were obtained from the gastric carcinoma patients who couldn't undergo operation due to radiological evidence of distant metastasis. Patient ages ranged from 28 to 82 years(mean =54). The number of male patients was 68 and female patients was 33. The histological examination of surgical specimens was assessed on paraffin sections stained by hematoxylin-eosin. Tumors were classified and graded according to WHO classification and we reviewed the site of tumor, Lauren's type of tumor, depth of invasion, lymphatic invasion, vein invasion and lymph node metastasis in 75 gastrectomy specimens. In the 26 patients, who have radiologic evidence of distant metastasis, informations about histologic type of tumor and grades of differntiation were only available. In nine cases, tissue block which contained metastasized tumor was available, and nm23 immunostaining at these sites was performend at the same time.

Immunostaining Procedure

All cases were studied with formalin-fixed, paraffin-embedded tissues. Five μm sections were examined using the avidina-biotin complex technique(ABC). All procedures were carried out at 40℃. Endogeneous peroxidase activity was blocked by treatment with 3 % hydrogen peroxide in absolute methanol. Normal rabbit serum(diluted as 1 : 5 in Tris buffered saline) was used as a blocking reagent. The used monoclonal antibodies were specific for human p53 protein(DO-7, Novocastra, 1 : 50 dilution, Newcastle upon Tyne, UK) and nm23(NDPK-A/nm23, Novocastra, 1 : 100 dilution, Newcastle upon Tyne, UK). The monoclonal antibody, DO-7 reacts with both the wild and mutant types of p53 protein. In the staining of p53, we used target unmasking fluid prior to application of the primary antibody. The sections were incubated with these antibodies for 90 minutes, followed by biotin-labelled rabbit immunoglobulin antimouse (DAKO) and streptavidin-biotin complex linked to alkaline phosphatase (DAKO). Slides were washed in TBS three times for 5 minutes each time. The color was developed in naphthol phosphate-new fuchsin solution (DAKO) after which the slides were slightly counterstained with hematoxylin and mounted with Crystal mount™(Biomeda Co., USA).

A section from a breast carcinoma with documented p53 overexpression was used as an external positive control. Negative controls consisted of a complimentary section from each tumor with substitution of nonimmune serum for the primary antibody. In the p53 staining, only nuclear positivity was analyzed and in the nm23, the cytoplasmic, nuclear, and membranous staining were analyzed seperately. The staining was considered negative when no cell was stained on the section and when a few cells or more were positive, the case was called positive.

Statistical Analysis

Statistical analysis was performed for each of the above criteria using chi-squared test or Fisher's exact test. Probability values less than 0.05 were considered to be statistically significant.

RESULTS

Expression of p53 protein in stomach carcinomas

The results of immunostaining of the p53 protein are summarized in Table 1. Among 101 specimens examined, 37(36.6 %) showed positive staining for the p53 protein and 64(63.4 %) showed no detectable p53 protein in tumor cells. The positive specimens, including each histologic subtype, showed overexpression of the p53 protein confined to nuclei of tumor cells(Fig. 1). normal gastric mucosa and infiltrating lymphocytes demonstrated no immunoreactivity for p53 staining. Comparison between p53 staining patterns in three histological subtypes of adenocarcinoma showed no significant differences. The intensity of immunostaining varies among tumor nuclei ; some were strong, some were weak and others were negative.

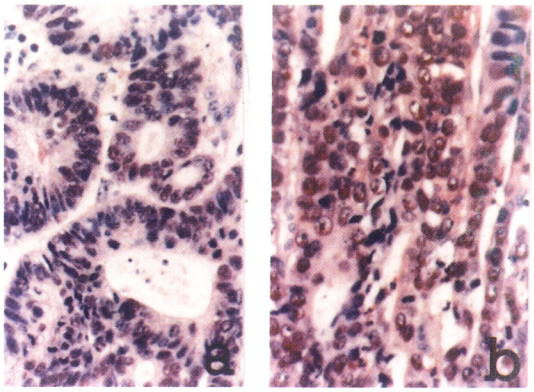

Fig. 1. Immunohistochemical detection of p53 protein in gastric well differentiated tubular adenocarcinoma(a) and poorlydifferentiated adenocarcinoma(b).

Correlation between p53 expression and clinicopathological features

The correlation between p53 expression and patient's age, sex, histologic type, grade, Lauren's type of tumor, depth of invasion, lymphatic invasion, vein invasion, lymph mode metastasis, and distant metastasis were tested and are summarized in Table 1. p53 positivity was observed in the cases of deeply

Table 1. p53 expression and clinicopatholigical variables in gastric cancers.

	Total No.	p53 positivity(%)	*P* value
No. of specimen	101	37(36.6)	
Sex			
Males	68	28(41.2)	0.174
Females	33	9(27.3)	
Tumor site			
Antrum(A)	38	12(31.6)	
Body(M)	32	8(25.0)	0.762
Antrum and body(AM)	5	1(20.0)	
Histological type			
tubular	87	32(36.8)	
signet-ring cell	12	5(41.7)	0.525
mucinous	2	0(0.0)	
Histological grade			
well	12	6(50.0)	
moderate	40	16(40.0)	0.390
poor	49	15(30.6)	
Lauren classification			
intestinal	51	22(43.1)	0.171
diffuse	50	15(30.0)	
Depth of invasion			
mucosa and submucosa	28	6(21.4)	
subserosa and serosa	38	10(26.3)	0.001
distant metastasis	35	21(60.0)	
Lymph node metastasis			
positive	39	15(38.5)	0.036
negative	36	6(16.7)	

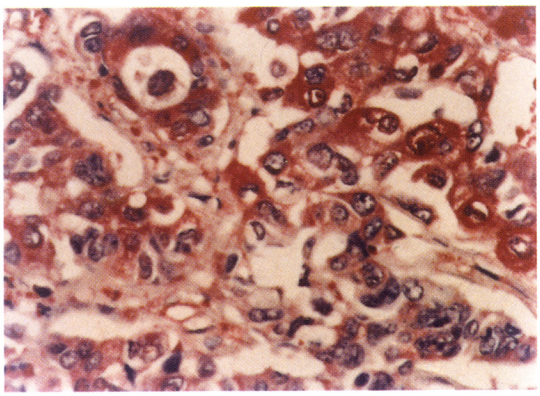

Fig. 2. Immunohistochemical detection of nm23 protein showing intensecytoplasmic positivity in moderately differentiated gastric carcinoma.

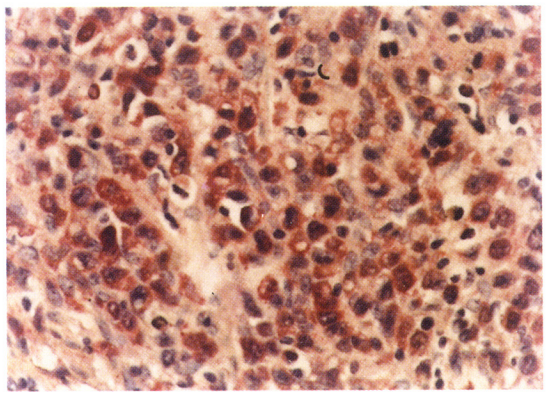

Fig. 3. Immunohistochemical detection of nm23 protein showing diffusenuclear staining in poorly differentiated gastric carcinoma cells. The infiltrating lymphocytes are negative for nm23.

invasive advanced carcinomas, carcinomas with frequent lymphatic invasion, tumor with lymph node metastasis and distant metastasis. After statistical analysis according to chi-squared test, p53 protein overexpression correlated with depth of invasion, lymphatic invasion, lymph node metastasis and distant metastasis.

Expression of nm23 in gastric carcinomas

In agreement with previous reports(Rosengard et al., 1989), staining was observed in the cytoplasmic (Fig. 2) and nuclear compartments of cells(Fig. 3), often with combined patterns. Often membranous staining was also observed(Fig. 4). Occasional normal gastric foveolar epithelial cells, stromal cells, and infiltrating lymphocytes demonstrated immunoreactivity for the nm23 protein. Positive staining within the tumor nuclei was homogenous and evenly distributed, although it sometimes showed accentuation around the nuclear border. In nine cases containing primary and metastatic tumor tissue, four cases of metastasized carcinoma were negative for nm23 though it was positive in the primary one. Interestingly, the remaining five cases showed more weak nm23 immunoreactivity in the metastatic site than in the primary tumor.

Correlation between nm23 and clinicopathological features

Thirty five cases had no staining reactivity at all. Out of 101, 62 cases(61.4 %) exhibited a cytoplasmic staining on most cells and 42 cases(41.6 %) had nuclear staining. In 16 of 101 cases(15.8 %), a mild to moderate membranous staining was observed in some cells. The cytoplasmic nm23 detection was decreased in the cases of gastric carcinomas with lymph node metastasis and distant metastasis. Reversely, the cytoplasmic nm23 detection was increased in the cases of gastric carcinomas without lymph node metastasis and distant metastasis. After statistical analysis according to chi-squared test, cytoplasmic nm23 expression was negatively correlated with lymph node metastasis($P<0.01$) and distant metastasis($P<0.01$). Like the results of cytoplasmic nm23,

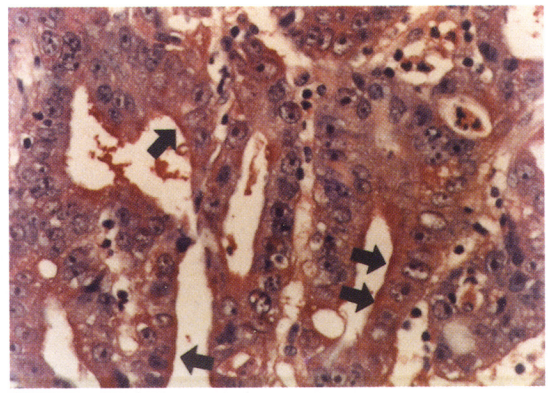

Fig. 4. Immunohistochemical detection of nm23 protein showing membranousstaining(arrow) in well differentiated gastric carcinoma.

Table 2. nm23 expression and clinicopatholigical variables in gastric cancers.

	Total No.	nm23 positivity(%)		
		cytoplasmic	nucleus	membranous
No. of specimen	101	62(61.4)	42(41.6)	16(15.8)
Sex				
Males	68	42(61.8)	31(45.6)	11(16.2)
Females	33	20(60.6)	11(33.3)	5(15.2)
Tumor site				
Antrum(A)	38	28(73.7)	20(52.6)	7(18.4)
Body(M)	32	19(59.4)	13(40.6)	6(18.8)
Antrum and body(AM)	5	3(60.0)	2(40.0)	2(40.0)
Histological type				
tubular	87	56(64.4)	37(42.5)	13(14.9)
signet-ring cell	12	6(50.0)	5(41.7)	3(25.0)
mucinous	2	0(0.0)	0(0.0)	0(0.0)
Histological grade				
well	12	9(75.0)	6(50.0)	3(25.0)
moderate	40	26(65.0)	16(40.0)	6(15.0)
poor	49	27(55.1)	20(19.8)	7(14.3)
Lauren classification				
intestinal	51	35(68.6)	22(43.1)	9(17.6)
diffuse	50	27(54.0)	20(40.0)	7(14.0)
Depth of invasion			*	
mucosa and submucosa	28	21(75.0)	18(64.3)	8(28.6)
subserosa and serosa	38	23(60.5)	16(42.1)	6(15.8)
distant metastasis	35	18(52.4)	8(22.9)	2(5.7)
Lymph node metastasis		*	*	*
positive	39	21(53.9)	11(28.2)	3(7.7)
negative	36	29(80.6)	24(66.7)	12(33.3)

* : Statistically significant

the nuclear nm23 expression showed negative correlation with depth of invasion($P<0.01$), lymphatic invasion($P<0.01$), lymph node metastasis($P<0.01$), and distant metastasis($P<0.04$). The membranous nm23 expression also revealed negative correlation with lymphatic invasion($P<0.02$), lymph node metastasis($P<0.01$) and distant metastasis($P<0.02$). And there was no statistical correlation between nm23 expression and p53 positivity($P<0.33$).

DISCUSSION

The p53 gene encodes for a 53-KDa nuclear phosphoprotein(p53) which is involved in cell cycle regulation. p53 operates a G1 arrest after DNA damage, which precludes its use as a template during replicative synthesis and thus avoids the fixing of genomic alterations in daughter cells(Kastan et al., 1991). Mutation of the p53 protein may represent the commonest genetic event in human malignancy(Hollstein et al., 1991). p53 gene inactivation is due to mutations generally localized in a highly conserved region(exons 5 to 9)(Iggo et al., 1990). These mutations result in the formation of a p53 protein with a longer half-life, which becomes detectable for the protein by immunohistochemistry(Iggo et al., 1990). There is generally good correlation between p53 mutations and p53 protein accumulation (Hurlimann and Saraga, 1994). A correlation between abnormal p53 expression and tumorigenicity and metastatic potential has been noted in colorectal cell lines(Trainer et al., 1988).

In gastric carcinomas, the frequency of p53 mutations varies from 4 to 64 %(Imazeki et al., 1992; Seruca et al., 1992; Yokozaki et al., 1992; Kakeji et al., 1993; Sasano et al., 1993). It is still controversial whether p53 mutations are associated with metastasis, a particular histologic type, tumor stage or prognosis(Hurlimann and Saraga, 1994). In our study, p53 positivity was 36.6 % in total. These results were very

similar to that of Hurlimann and Saraga(1994). In early gastric carcinomas, the p53 detection rate was 21.4 % and in advanced cases, p53 positivity was 45.5 %. This results are highly consistent with that of Uchino et al.(1992). But unlike the study of Hurlimann and Saraga(1994), there was no statistical significance in p53 positivity between diffuse and intestinal histologic types in our study. There was no relationship between p53 protein accumulation and sex, tumor site, histologic grade, and Lauren classification. These previously described results were consistent with that of Uchino et al.(1992). But contrary to the study of Uchino et al.(1992), who performed the study bearing some resemblance to ours, the p53 expression in our study was statistically correlated with the depth of invasion, degree of lymphatic invasion, lymph node metastasis and distant metastasis. These results are very similar to that of Martin et al.(1992) and Hurlimann and Saraga(1994). As the depth of invasion deepened and the lymphatic invasion was more frequently observed, the p53 expression was more frequently detected. In our study, only 21.4 % of early gastric carcinomas expressed p53 protein whereas 42.5 % of advanced and metastatic carcinomas overexpressed p53 protein. Out of 34 distant metastasized gastric carcinomas, 21 specimens(61.8 %) expressed p53 and out of 67 localized gastric carcinomas with or without lymph node metastasis, only 16 specimens(23.9 %) expressed p53. These results are very similar with that of Kakeji et al.(1993). It is not clear whether p53 mutation is an early or late event(Correa, 1992). The finding of p53 mutations in gastric dysplastic cells or in early carcinoma is a strong argument for an early effect(Correa, 1992). On the other hand, the presence of p53 has been preferentially described in advanced stages of gastric carcinoma like ours(Tamura et al., 1991 ; Yamada et al., 1991) Increased expression of p53 protein in advanced, lymph node metastasized and distant metastasized gastric carcinomas intensely support the argument that p53 mutation is an late event in gastric carcinogenesis and p53 expression may have relation to metastasis and poor prognosis in gastric carcinoma. The prognosis of gastric carcinoma depends mainly on depth of invasion and the extent of nodal and distant metastasis at the time of diagnosis : histologic type has no prognostic significance(Cotran et al., 1994). In this aspect, our results strongly suggest that expression of p53 does influence tumor behavior and may be clinically useful for getting some information on the metastatic potential of gastric carcinoma, which was observed by other authors, too(Martin et al., 1992 ; Kakeji et al., 1993).

Nonmetastatic (nm)23 gene expression correlates inversely with metastatic potential in several rodent tumor model systems as well as in human infiltrating breast and hepatocellular carcinomas(Lakso et al., 1992). In human tissues two nm23 genes were identified, nm23-H2 (Stahl et al., 1991), that exhibits an 88 % homology to the classic nm 23 gene, nm 23-H1. Reduced expression of the nm23 gene is implicated in high metastatic potential in a variety of malignancies(Nakayama et al., 1993). The actual mechanism by which nm23 affects metastatic or invasive properties is unknown. The nm23-H1 codes for a gene product that is almost homologous in the amino acid sequence with NDP kinase A in human erythrocytes(Gilles et al., 1991). NDP kinase is known to be a microtubule associated protein and is speculated to take part in microtubule assembly and disassembly, influencing the cytoskeleton status(Hirayama et al., 1991). Thus, it could be predicted to affect the abilities of the cells to move or adhere, the qualities that partially explain the metastatic potential of cancer cells(Kodera et al., 1994). In gastric cancer, it was reported that nm23 gene expression is higher in primary cancer tissues than in matched mucosa (Nakayama et al., 1993). However, immunostatining in autopsy cases of gastric cancer has revealed that metastatic liver tumors exhibit weaker immunoreactivity than do primary tumors and this might suggest the role of nm23 gene as a metastasis suppressor gene (Kodera et al., 1994). However the efforts to know the relation between nm23 and metastasis are mainly confined to breast and colorectal carcinomas(Kodera et al., 1994). So we studied the expression rate and role of nm23 protein in the metastasis of gastric carcinomas and their relation to clinicopathological parameters. In our cases, the nm23 positivity was 46.3 % and nm23 expression was negatively correlated with depth of invasion, lymph node metastasis and distant metastasis. Nakayama et al.(1993) examined the loss of heterozygosity(LOH) of nm23 gene by Southern blotting, nm23 mRNA expression by Northern blotting and nm23 protein expression by immunohistochemistry in both primary and metastatic tumors. They could not find any correlation between clinicopathological features and LOH of nm23 gene and nm23 mRNA expression, but found reduction of nm23 immunoreactivity in the metastatic tumor of

regional lymph nodes, compared to the primary tumor. These results suggest that nm23 overexpression is linked with development of gastric carcinomas and the decrease in expression of nm23 participate in metastasis(Kodera et al., 1994). In nine cases of which specimen containing primary and metastatic tumor tissue in a organ such as liver, colon and pancreas, nm23 immunostaining was performend at the same time in our study. In four cases, nm23 which was positive in the primary gastric carcinoma region was negatively stained in the metastatic site. Interestingly, the remaining five cases showed weaker nm23 immunoreactivity in the metastatic site than in the primary tumor. These results are partially consistent with that of Nakayama et al.(1993). These results support the previous assumption proposed by Kodera et al.(1994), that the decrease in expression of nm23 participates in metastasis.

If our result is confirmed by a follow-up, the availability of a marker, that can predict the risk of metastasis would greatly improve the care of patients with gastric cancer.

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