

Prognostic significance of metastasis-suppressor gene NM23 in gastric carcinoma

Metastaz supressör genlerden NM23'ün mide kanserindeki prognostik önemi

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Abstract

Aim: Metastasis is critical in the prognosis of gastric cancer patients and in deciding on treatment strategies. Therefore, studies have concentrated on metastasis suppressor genes. This study aimed to identify the characteristics of NM23 expression in gastric cancer and to investigate its anti-metastatic and prognostic significance.

Methods: Ninety patients who underwent surgery for gastric cancer between January 1, 2009 and January 1, 2010 were included in this study. Immunohistochemical staining was applied to specimen sections. The results of the immunohistochemical staining were evaluated with the normal gastric mucosa adjacent to the tumor. The degree of NM23 staining of tumor cell compared to the normal tissue was evaluated in two groups of "negative-weak staining" and "strong staining". Both groups were analyzed for lymph node metastasis, vascular invasion, perineural invasion, degree of tumor differentiation, tumor stage and distant metastasis.

Results: Among 58 patients with lymph node metastasis, 70.7% showed negative-weak staining and 29.3% showed strong staining. Among 32 patients with no lymph node metastasis, 40.6% had negative-weak staining and 59.4% had strong staining. Decrease in NM23 expression was shown to cause an increase in lymph node metastasis ($P=0.01$). Furthermore, while there was no statistical significance, the rates of distant metastasis, degree of poor differentiation, tumors in stage III and stage IV were apparently higher in the negative-weak staining group. These results were in line with the anti-metastatic property of NM23.

Conclusion: This study showed that NM23 expression had anti-metastatic properties for lymph node metastasis in gastric cancer patients.

Keywords: Gastric carcinoma, Immunohistochemical staining, NM23

Öz

Amaç: Metastaz, mide kanserli hastaların prognozunda ve tedavi stratejilerinin belirlenmesinde kritik öneme sahiptir. Bu nedenle metastaz baskılayıcı genler üzerine çalışmalar yoğunlaşmıştır. Bu çalışmada da, mide kanserlerinde NM23 ekspresyonunun özelliklerinin belirlenmesi, anti-metastatik ve prognostik öneminin araştırılması amaçlanmıştır.

Yöntemler: 1 Ocak 2009 - 1 Ocak 2010 tarihleri arasında mide kanseri nedeniyle opere edilen 90 hasta çalışmaya alındı. Spesmen kesitlere immünohistokimyasal boyama uygulandı. İmmünohistokimyasal boyama sonuçları, tümöre bitişik normal gastrik mukozaya ile beraber değerlendirildi. Normal dokuya göre tümör hücrelerinin NM23 ile boyanma derecesi: "negatif-zayıf boyanma" ve "kuvvetli boyanma" olmak üzere iki grup olarak değerlendirildi. Her iki grup, lenf nodu metastazı, vasküler invazyon, perinöral invazyon, tümör diferensiyasyon derecesi, tümör evresi ve uzak metastaz için değerlendirildi.

Bulgular: Lenf nodu metastazı bulunan 58 hastanın %70,7'si negatif-zayıf boyanma gösterirken %29,3'ü kuvvetli boyanma özelliği gösterdi. Lenf nodu metastazı bulunmayan 32 hastanın ise %40,6'sı negatif-zayıf boyanma özelliğine, %59,4'ü kuvvetli boyanma özelliğine sahipti. NM23 ekspresyonundaki azalmanın, lenf nodu metastazındaki artışa neden olduğu gösterildi ($P=0,01$). Ayrıca istatistiksel anlamlılık bulunmamakla beraber, negatif-zayıf boyanma gösteren grupta, uzak metastaz, kötü diferensiyasyon derecesi, evre III ve evre IV tümörlerin oranı belirgin olarak daha yüksekti. Bu bulgular da NM23'ün anti-metastatik özelliğini destekledi.

Sonuç: Bu çalışma NM23 ekspresyonunun, mide kanserli hastalarda, lenf nodu metastazı için anti-metastatik özelliğe sahip olduğunu gösterdi.

Anahtar kelimeler: Mide kanseri, İmmünohistokimyasal boyama, NM23

Introduction

Gastric carcinoma is aggressive, and one of the most common gastrointestinal cancers [1]. Its incidence varies geographically, and its prevalence is not uniform. The frequency of gastric carcinoma is particularly higher in Japan and Colombia [2,3]. Despite increasing resectability rates and decreasing incidence in Western societies starting from the second half of the twentieth century, gastric carcinoma remains the second global leading cause of the cancer-related deaths [4].

Metastasis is a critical factor in the prognosis of cancer patients and plays an important role in mortality. Studies have focused on this area to define novel therapeutic strategies. The discovery of a new class of genes, which are metastasis-suppressor genes, has attracted a lot of attention. The first identified metastasis suppressor gene is NM23 [5]. Murine NM23 cDNA was discovered using differential colony hybridization in murine K1735 melanoma cell lines. mRNA and protein levels of NM23 were studied in numerous model systems and its decreased expression was detected in highly metastatic samples [6]. In humans, NM23 gene family (also known as NME genes) comprises 10 genes. It is located at 17q21, and encodes nucleoside diphosphate kinase A. So far, it was clearly shown that NM23-H1 is a critical regulator of the signaling networks that play a role in local invasion and the adhesion of cancer cells in primary tumors [7]. However, the mechanism by which NM23 suppresses tumor metastasis is still not understood. Despite studies indicating that NM23 is anti-metastatic, there are other studies reporting contradictory results. A definitive conclusion is yet to be reached.

In the light of previous research, we thought that the NM23 gene expression can help with the prediction of metastatic diseases. If metastatic disease can be predicted through NM23, patients with a high risk of metastasis can undergo neoadjuvant chemotherapy to decrease recurrence and improve survival rates. For this purpose, the study aims to investigate the antimetastatic properties of NM23.

Materials and methods

This study was approved by the Ethics Committee of Erciyes University School of Medicine. This cohort consists of ninety patients (male 67, female 23) who were diagnosed with gastric carcinoma and operated at Department of General Surgery of Erciyes University School of Medicine between January 1, 2009 and January 1, 2010. The demographic data of patients, i.e. gender, age and tumor localization, tumor size, operation, surgical margin, TNM stage and differentiation, presence of local, vascular and perineural invasion, lymph node involvement, distant and local organ metastasis were recorded.

Patient selection

Based on the pathology, those who are positive for lymph node metastasis were included in the metastasis group, and those who are negative for lymph node metastasis were included in the non-metastatic group. Although paraffin samples obtained from distant metastatic sites such as the liver or lymph nodes were stained with NM23, immunohistochemical staining was performed on the gastric tumor to evaluate prognostic factor

(lymph node metastasis, vascular invasion, perineural invasion, local invasion, degree of differentiation and distant metastasis).

Immunohistochemistry (IHC)

General IHC Protocols database was used for NM23 expression analysis in pathological samples. Samples were fixed with 10% formaline, prepared with routine processing, cut in 3 to 5-micron slices and embedded in paraffin. Endogen peroxidase activity was blocked with hydrogen peroxide to minimize non-specific staining and ground dyeing. The samples were washed for 10 minutes in phosphate buffered saline solution. As the primary antibody, NM23-H1 (1/50 dilution) (37.60) (sc-56928, lot#L2107 mouse IgG2a supernatant stored at -20°C, 250 microliters Santa Cruz Biotechnology) was used. This antibody is similar to the NM23-H1 gene product and it increases when NM23-H1/NDP kinase A is present.

Streptavidin-biotin peroxidase method was used for immunohistochemical staining (SPLink HRP Detection Bulk Kit for Mouse and rabbit antibodies streptavidin-biotin kit). Results of immunohistochemical staining were evaluated together with the normal gastric mucosa adjacent to the tumor. Depending on the degree of staining of the tumor cell with NM23 compared to the normal tissue, immune staining was classified into two groups: "negative-weak staining" and "strong staining" (Figure 1).

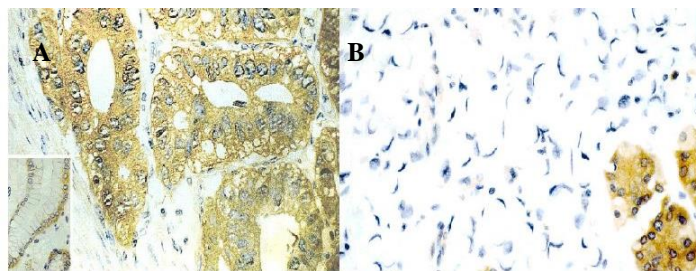


Figure 1: Immunohistochemical staining with NM23 (A: Normal gastric mucosa, strong cytoplasmic staining with NM23, B: Signet cell gastric cancer, Negative staining with NM23)

Statistical analysis

Statistical analyses were performed using SPSS version 15 software. The conformity of the variables to normal distribution was examined using visual (histogram and probability graphs) and analytical (Kolmogorov-Smirnov/Shapiro-Wilk tests) methods. In descriptive analyses, variables with normal and non-normal distribution were expressed as mean and median, respectively. To assess the prognostic significance and anti-metastatic properties of NM23 in gastric carcinomas, those with no NM23 staining (negative) and with focal or diffuse weak staining were included in the "negative-weak staining group" (Group 1), and those with focal or diffuse strong staining with NM23 were included in the "strong staining group" (Group 2). The statistical significance of differences between Groups 1 and 2 in terms of lymph node metastasis, vascular invasion, perineural invasion, degree of tumor differentiation, tumor stage and distant metastasis was determined by Chi-square or Fisher tests. A *P*-value less than 0.05 was considered statistically significant.

Results

Table 1 presents the demographic data of the patients. 67 patients are male and 23 are female. Male/female ratio was 2.88 and the median age of the patients was 63 (range, 32-83)

years. No statistically significant difference was found between the groups in terms of age and gender (Table 1).

Table 1: The distribution of general characteristics based on patient and tumor

	n	%
Gender		
Male	67	74.4
Female	23	25.6
Tumor location		
Antrum	63	70
corpus	13	14.5
Cardia	8	8.9
Linitis plastica	6	6.6
Tumor size (cm)		
0-5	29	32.2
5-10	46	51.1
>10	15	16.7
Operation		
Radical subtotal gastrectomy	26	28.9
Total radical gastrectomy	64	71.1

70.7% of 58 patients with lymph node metastasis showed negative-weak staining and 29.3% showed strong staining. 40.6% of 32 patients with no lymph node metastasis had negative-weak staining and 59.4% had strong staining. Group 1 had more lymph node metastasis than group 2 ($P=0.01$). No statistically significant difference was found between the two groups regarding vascular invasion ($P=0.23$), perineural invasion ($P=0.36$), degree of tumor differentiation ($P=0.54$), distant metastasis ($P=0.39$) and tumor stage ($P=0.24$) (Table 2).

Table 2: Evaluation of prognostic variables with NM23 according to staining intensity

		Negative-weak staining group (Group 1)	Strong staining group (Group 2)	P-value
		n	n	
Lymph node metastasis	Positive	41	17	0.01
	Negative	13	19	
Vascular invasion	Positive	35	18	0.23
	Negative	19	18	
Perineural invasion	Positive	26	13	0.36
	Negative	28	23	
Degree of tumor differentiation	Well-differentiated	12	11	0.54
	Moderately-differentiated	15	11	
	Poorly-differentiated	27	14	
Stage	I	11	12	0.24
	II	9	9	
	III	9	3	
	IV	25	12	
Distant metastasis	Positive	17	8	0.39
	Negative	36	29	

While there was no significant difference between degree of tumor differentiation and groups ($P=0.54$), NM23 staining in poorly differentiated tumors was observed to be 65.9% and 34.1% for Group 1 and Group 2, respectively. Rate of negative-weak staining was significantly higher in poorly differentiated tumors. These rates supported the anti-metastatic property of NM23. There was no statistically significant difference between Group 1 and Group 2 in terms of distant metastasis. However, our findings supported the anti-metastatic property of NM23. Negative-weak staining was observed in 68% of the patients with distant metastasis, and 32% of the patients showed strong staining. Likewise, negative-weak staining was at 64.6%, and strong staining was at 32.4% in stage IV tumors. In stage III tumors, these ratios were 75% and 25%, respectively. These findings were deemed to be supporting the anti-metastatic property of NM23 expression (Table 2).

Discussion

Although there has been a decrease in mortality in the last 60 years, gastric carcinomas are still the most common cancers and the most common causes of mortality [8]. Metastasis

is a critical factor in the prognosis of cancer patients [9]. The first identified metastasis suppressor gene is NM23. Decreased NM23 expression has been associated with potentially increased metastasis in various cancers, i.e. breast cancer, gastric cancer, melanoma [10-14]. In lung and pancreatic cancer, increased NM23 expression is associated with poor prognosis [15]. In the literature, NM23 is thought to play different roles in different tissues. Some studies show that NM23 expression in gastric carcinoma doesn't predict survival and liver or lymph node metastasis [16-18]. In this study, we found that the decrease in NM23 expression caused an increase in lymph node metastasis.

Lee et al. [19] have retrospectively analyzed 841 patients who underwent gastrectomy due to gastric carcinoma. Contrary to our results, it was shown that increased NM23 expression is correlated with poor prognosis. Consistent with the results of Lee et al. [19], Müller et al. [20] have reported that there is a positive correlation between increased NM23 expression and poor prognosis and lymphatic vessel invasion.

We observed that the decrease in NM23 expression led to increased lymph node metastasis. Consistent with our study, Kodera et al. [21] showed that decreased NM23 expression was associated with increased lymph node metastasis. They found no clinically significant correlation between decreased NM23 expression and distant metastasis.

In their study on NM23 expression, Liu et al. [22] have reported that decreased NM23 expression is associated with increased lymph node involvement. On this subject, Yu et al. [23] have noted that there is a correlation between decreased NM23 expression and increased distant metastasis. Our research results coincide with the study of Liu et al. [22]. We could not observe statistical significance with regards to distant metastasis. However, our findings support the argument of Yu et al. [23] that decreased NM23 expression in gastric cancers may lead to increased distant metastases.

No result concerning the anti-metastatic property of NM23 could be reached in some studies on NM23 in gastric cancer. Monig et al. [24], who investigated the correlation between NM23 and prognosis, could not detect any correlation between NM23 expression and distant metastasis, lymph node involvement or prognosis in gastric carcinomas. Similar results were obtained by Radovic et al. [4] who suggested that there was no significant correlation between clinicopathologic variables and NM23 expression. The study by Yang [25] also corroborated these results and concluded that there was no correlation between NM23 expression and metastases, also noting that NM23 can play a role in tumor pathogenesis.

Limitations

The number of patients and the fact that it was a single-center study proved to be the limitations of our study.

Conclusion

This study demonstrated that NM23 expression had anti-metastatic properties for lymph node metastasis. In addition, findings supporting the anti-metastatic property of NM23 expression were obtained in terms of distant metastasis, degree of tumor differentiation and tumor stage. Nevertheless, there are still conflicting findings regarding the anti-metastatic properties of NM23 gene expression. It is therefore thought that further research is needed.

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