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High nucleostemin expression has a favorable prognostic effect on gastric carcinomas

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Background/aim: Nucleostemin is a nuclear protein that maintains stem cell features and plays a role as a cell proliferation marker. It also participates in cell cycle regulation by interfering with other intracellular proteins. Recent reports have indicated that this protein plays a role in ribosomal biogenesis and genome protection. High expression level of nucleostemin has been reported in some cancer patients. However, the importance of nucleostemin in gastric cancer needs to be addressed. The aim of this study was to investigate nucleostemin expression in gastric cancer and the effects of this expression on prognosis.

Materials and methods: Nucleostemin expression was assessed in 103 patients with gastric carcinomas via immunohistochemistry. Subsequently, relationships between nucleostemin expression and clinicopathological features and prognosis were evaluated.

Results: In this study, there were 33 and 70 cases involving high and low nucleostemin expression, respectively. Nucleostemin expression was negatively correlated with lymphovascular invasion, the number of metastatic lymph nodes, extracapsular extension, and T stage. Disease-free survival and overall survival were markedly longer in patients with high nucleostemin expression.

Conclusion: We suggest that nucleostemin is a favorable prognostic marker for gastric cancer patients. Our results are in conflict with prior studies. The prognostic effect of nucleostemin in gastric cancer remains to be solved.

Key words: Gastric carcinoma, nucleostemin, prognosis

1. Introduction

Globally, gastric cancer is the fourth most common cancer in men, the fifth most common cancer in women, and among the five leading types of cancer with respect to causing death (1). During early stages of gastric cancer, nonspecific symptoms such as nausea, vomiting, and fatigue delay diagnosis. Treatment provides limited benefits in cases diagnosed at an advanced stage (2). Recently, studies to reveal the pathogenesis of stomach cancer have intensified, and investigations have focused on targeting therapeutic agents at the molecular level.

Nucleostemin (NS), a GTPase primarily residing in the nucleolus, was recently found to be essential in the late step of pre-RNA processing of the ribosomal assembly and overall protein synthesis (3,4). NS is expressed in embryonic stem cells, central nervous system stem cells, primitive bone marrow cells, and the testis. NS is also necessary for the proliferation of human stem cells and certain cancer cells (5). Due to interactions with a range of molecules, such as the tumor-suppressing protein p53, NS is thought to play an important role in cellular self-renewal, cell cycle regulation, apoptosis, and cell proliferation (6,7). Recently, the protective effects of NS on DNA and telomeres have been revealed (8,9).

However, after nearly 15 years of research, the functional properties of the NS protein have not been fully described yet. Dai et al. reported that NS binds to MDM2 (mouse double minute 2) and inhibits the degradation of p53 (10). p53 remains at low levels in normal unstressed cells due to MDM2. High NS levels eliminate the inhibitory effect of MDM2 on p53, leading to the suppression of cell proliferation (10). On the other hand, in cultured neural stem cells, depletion of nucleostemin triggers DNA damage due to replication and spoils self-renewal, whereas overexpression of nucleostemin has a protective effect against hydroxyurea-dependent DNA damage (11).

NS has been frequently found upregulated in various proliferating cell types, including cancer cells and stem cells, and was thus designated as a cell proliferation marker as well as a marker for poor prognosis in cancer patients (3,12,13). High NS expression level was found to be associated with aggressive behavior and worse

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prognosis in squamous cell carcinoma of the cervix, renal cell carcinoma, prostatic carcinoma, esophageal carcinoma, and breast carcinoma (12–16). In our study, we sought to reveal the expression of NS in gastric carcinoma, identify correlations between NS expression and clinicopathological parameters, and assess the effects of NS expression on prognosis.

2. Materials and methods

2.1. Patients and tumor specimens

The study included a total of 103 gastric cancer cases involving gastric resection that were diagnosed between 2006 and 2015 at the Pathology Department of Eskişehir Osmangazi University. Patients' demographic characteristics and histopathological prognostic parameters were recorded. Overall and disease-free survival times for the included cases were obtained from the Medical Oncology Department.

2.2. Immunohistochemistry

Paraffin blocks that best reflected tumor morphology were studied via immunohistochemical staining for NS (ab70346 – Abcam, Eugene, OR, USA). Sections with a thickness of 4 μm were obtained from these paraffin blocks, and slides were deparaffinized. Immunoperoxidase staining was then completed using an automatic staining machine (the Ventana BenchMark XT Automated IHC/ISH slide staining system) in accordance with the manufacturer's instructions. Chromogenic diaminobenzidine (DAB) was used for signal detection, and cells were counterstained with Harris hematoxylin. Negative controls were incubated with the same concentration of immunoglobulin (IgG1; Dako, Ely, UK) instead of the primary antibody. The positive controls were testicular seminoma specimens. Nuclear staining was regarded as a positive result. Staining intensity was evaluated as 1+ (mild), 2+ (moderate), or 3+ (intense). NS expression level was evaluated using the following scale: 0 (no stain), 1+ (<25% of tumor cells), 2+ (25%–75% of tumor cells), and 3+ (≥75% of tumor cells). The total expression score was obtained by multiplying the corresponding scores for staining percentage and intensity.

2.3. Statistical analysis

Associations between NS expression and demographic data and histopathological parameters were assessed using chi-square tests. A post hoc test (a correction) was applied for crosstabs with more than three groups. The critical staining score was examined by the receiver operating characteristic (ROC) curve. Values below this score were regarded as low expression, and values above this threshold were regarded as high expression. Survival analysis was based on the Kaplan–Meier method, and statistical significance was assessed via the log-rank test. $P < 0.05$ was considered significant for all statistical analyses.

3. Results

The patients included in the study were between 26 and 87 years of age (mean: 60.94 years). There were 68 male and 35 female cases. Patients were followed for a mean of 28 months (4–119 months). NS staining intensity was mild, moderate, and intense in 40, 52, and 11 cases, respectively (Figures 1–3). The staining percentage was less than 25% in 22 cases, 25%–75% in 50 cases, and above 75% in 31 cases. The staining scores ranged from 0 to 9. Statistical analyses indicated that the critical staining score was 4.5 according to ROC curves. As a result, 33 cases involved high and 70 cases involved low NS expression.

There was an inverse correlation between NS expression and number of metastatic lymph nodes. Cases with high NS expression involved fewer metastatic lymph nodes ($P = 0.004$). Similarly, extracapsular tumoral extension in metastatic lymph nodes was less in cases with high NS expression than in cases with low NS expression ($P = 0.044$). High NS expression was also inversely correlated with lymphovascular invasion, T stages, and clinical stages ($P = 0.014$, $P = 0.032$, and $P = 0.014$, respectively). Clinicopathological parameters and NS expression levels for the included patients are presented in Table 1. In survival analysis, cases with high NS expression exhibited markedly longer disease-free and overall survival durations ($P = 0.009$ and $P = 0.024$, respectively) (Figures 4 and 5). Multivariate analysis revealed that NS expression was not an independent prognostic parameter for disease-free survival or overall survival ($P = 0.639$ and $P = 0.811$, respectively) (Table 2).

4. Discussion

Gastric cancer continues to remain among the leading types of cancers with respect to causing death. Despite developments in diagnosis and treatment methods,

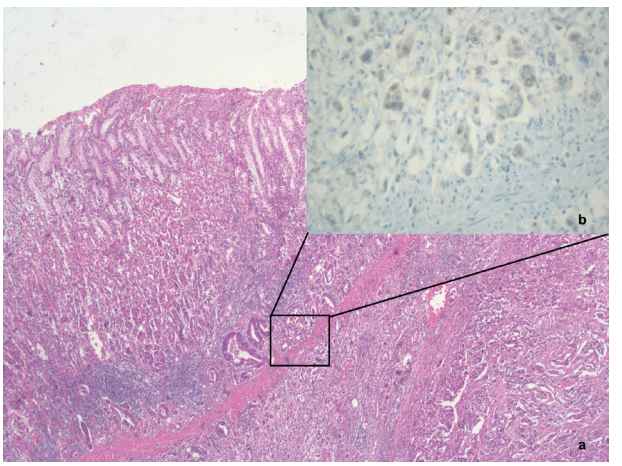

Figure 1. Poorly differentiated adenocarcinoma (a) (H&E, 40×) and low nucleostemin expression (b) (ab70346, 400×).

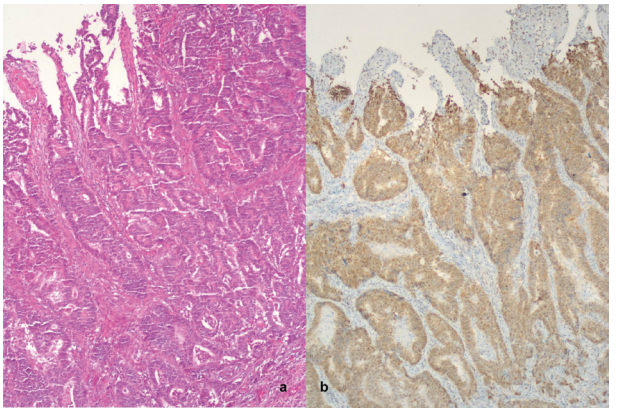

Figure 2. Gastric adenocarcinoma (a) (H&E, 100×) and moderate nucleostemin expression (b) (ab70346, 100×).

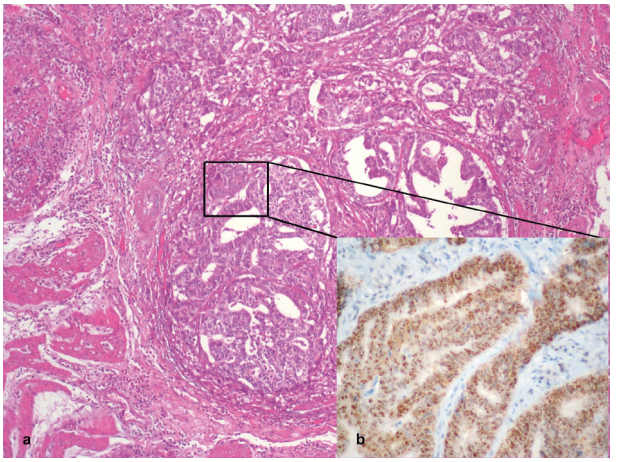

Figure 3. Adenocarcinoma with muscularis propria invasion (a) (H&E, 100×) and high nucleostemin expression (b) (ab70346, 100×).

the prognosis for gastric cancer continues to worsen. Currently, target-oriented treatments are of particular interest, and the determination of prognostic molecules for use as targets is gaining in importance. Recently, the role of NS as a proliferation indicator has come to the forefront. The expression level of NS in certain tumors has attracted research attention to this protein due to its probable contributions to tumor development and potential use as a prognostic parameter.

NS expression and its correlation with prognosis in different cancer types have been researched. Liu et al. demonstrated NS expression in esophagus, stomach, pancreas, ovary, and urine bladder cancer by RT-PCR (17). In cervical cancer, there were higher NS expressions than in cervical intraepithelial neoplasia. Similarly, cervical intraepithelial neoplasia had higher NS expression than normal cervical epithelium (12). Higher NS expressions were detected in poorly differentiated non-small cell lung cancer than in well-differentiated non-small cell lung cancer (18). Kobayashi et al. studied the prognostic effect of NS in invasive breast cancers and reported that cases with positive NS expression exhibited markedly shorter disease-free survival (16). The overexpression of NS in oral squamous cell carcinoma was correlated with advanced T and N stage, and worse prognosis (19). In these reports higher NS expression was associated with malignancy, aggressive behavior, and worse prognosis. In our study, in contrast to these prior results, higher NS expression was observed in tumors at earlier T stages ($P = 0.032$) and clinical stages ($P = 0.014$). Similarly, higher NS expression was inversely correlated with lymph node metastasis rate ($P = 0.004$) and extracapsular tumoral extension in metastatic lymph nodes ($P = 0.044$). Additionally, we found that cases with high NS expression involved significantly longer disease-free ($P = 0.009$) and overall ($P = 0.024$) survival times.

There were few studies on NS expression in gastric carcinoma. Asadi et al. identified high NS levels in high-grade carcinomas by RT-PCR (20). They proposed that high NS levels may have a causative role in the tumorigenesis and/or progression of stomach carcinoma. Additionally, they stated that NS might be used as a potential marker for diagnosis, molecular classification, and molecular treatment. Wu et al. reported that high NS expression was correlated with lymph node metastasis, T stage, and shorter survival times (21). These results are also opposite to our findings. In contrast to prior studies, we concluded that high NS expression is associated with favorable prognosis and longer survival times in gastric carcinoma. High NS expression was associated with increased cellular proliferation potential and tumor malignancy during cancer development (4,6,7). However, despite the association between NS levels and cellular proliferation potential, the influence of NS expression and activity on tumor development remains poorly understood and controversial. NS protein levels were found to be high in many cancers and in rapidly proliferating stem cells; both overexpression and depletion of NS cause cell cycle arrest in p53 wild-type U2OS cells (3,6,10). It has been reported that imbalanced NS levels result in a growth inhibitory effect by regulating the tumor suppressor activity of p53 (10).

Numerous proteins such as P53, MDM2, TRF1 (telomeric repeat binding factor 1), ARF, and RSL1D1 (ribosomal L1 domain containing 1) have been shown to interact with NS in mammals (22). Different responses to NS/p53 loss of normal and cancer cells were detected. Mechanisms that drive differential responses of cancer cells to disruption of NS/p53 may be related to other

Table 1. Clinicopathological features and nucleostemin expression levels.

			Nucleostemin expression		
		n (%)	Low	High	P-value
Age, years	<60	48 (46.6)	33	15	0.873
	≥60	55 (53.4)	37	18	
Localization	Proximal	31 (30.1)	22	9	0.668
	Distal	72 (69.9)	48	24	
Tumor dimension	<5 cm	43 (41.8)	27	16	0.341
	≥5 cm	60 (58.2)	43	17	
Grade	1	12 (11.7)	5	7	0.104
	2	35 (34)	24	11	
	3	56 (54.3)	41	15	
Perineural invasion	(–)	23 (22.3)	12	11	0.066
	(+)	80 (77.7)	58	22	
Lymphovascular invasion	(–)	25 (24.3)	12	13	**0.014**
	(+)	78 (75.7)	58	20	
Metastatic lymph nodes	(–)	24 (23.3)	9	15	**0.004**
	1–2	19 (18.4)	15	4	
	3–6	23 (22.3)	17	6	
	> 6	37 (35.9)	29	8	
Extracapsular extension	(–)	57 (55.3)	34	23	**0.044**
	(+)	46 (44.7)	36	10	
T stage	1	11 (10.7)	7	4	**0.032**
	2	10 (9.7)	4	6	
	3	43 (41.7)	26	17	
	4a	36 (35)	30	6	
	4b	3 (2.9)	3	0	
Clinical stage (TNM)	1a	9 (8.7)	5	4	**0.014**
	1b	4 (3.9)	1	3	
	2a	14 (13.6)	5	9	
	2b	12 (11.7)	8	4	
	3a	19 (18.4)	14	5	
	3b	24 (22.3)	20	3	
	3c	22 (21.4)	17	5	

Bolded entries are statistically significant P-values.

cancer-related mutations, such as inactivation of p16 in cancer cells, while continuing to be case-based. Moreover, beyond NS and p53, they may develop different adaptive responses that alter the expression of genes (23).

It has not yet been clarified whether aberrant NS levels have a role independent from p53 in controlling cell proliferation and growth. Lo et al. proposed that NS can act as an alternative tumor suppressor protein, protecting the cell from the effects that induce growth (24). They revealed that fluctuation in NS protein levels alters the alternative reading frame (ARF) protein, a cell cycle checkpoint, and stability. Decreasing the level of the NS protein reduces ARF protein levels and shortens its stability. Elevation of the ARF level caused by high levels of NS triggers G1 cell cycle arrest (24). These studies indicate that the behavior of NS as an oncogene or a tumor

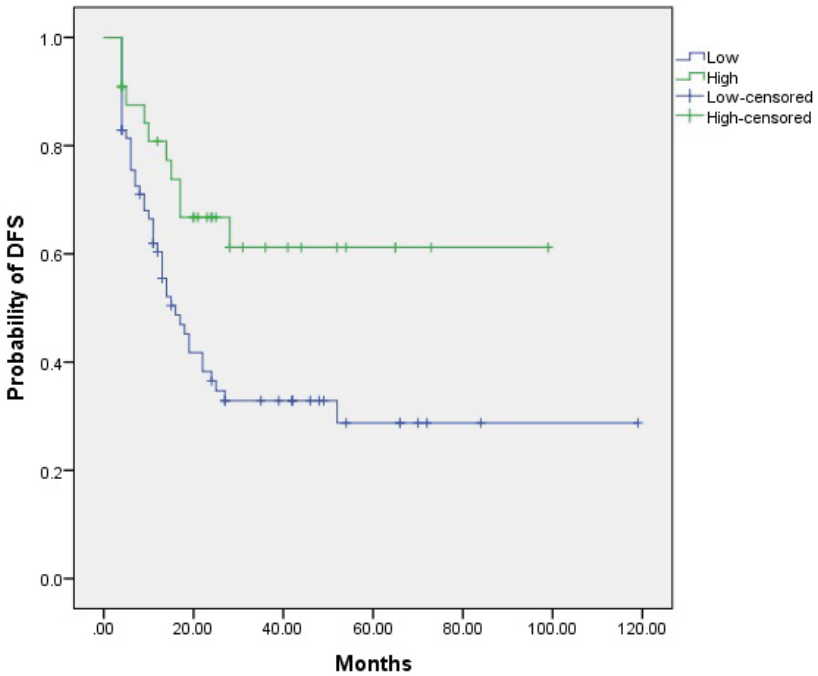

Figure 4. The association between nucleostemin expression and disease-free survival.

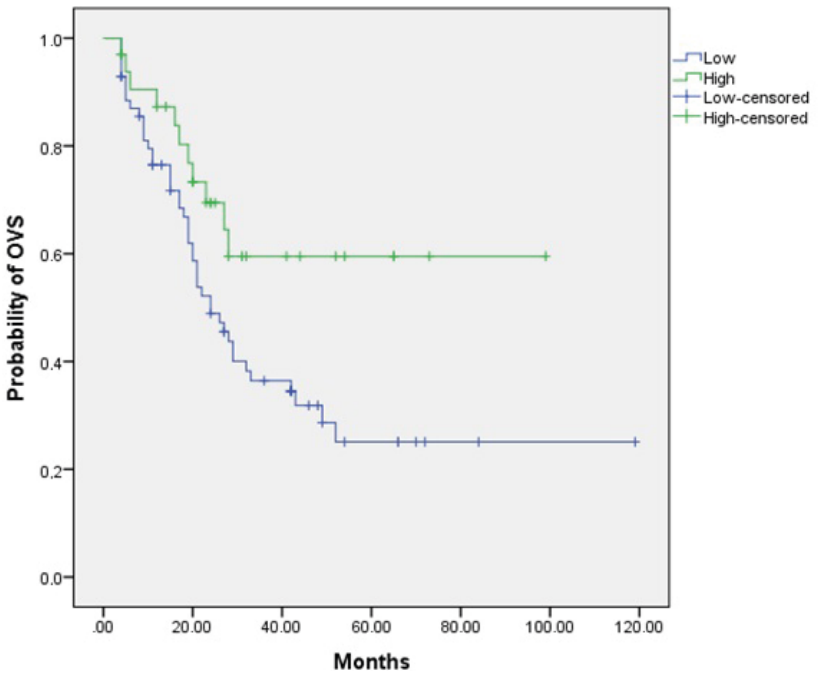

Figure 5. The association between nucleostemin expression and overall survival.

suppressor protein may depend on the genetic basis of the cells. The link between ribosomal biogenesis and the p53-ARF signaling pathway serves as an internal control mechanism that can stop cell growth or induce cell death in cells that proliferate with abnormal ribosome biosynthesis (25,26). However, the accumulation of genetic instability in cancer development may lead to the loss of p53 or ARF tumor suppressor functions (27). While overexpression of NS in the presence of p53 or ARF may lead to cell cycle arrest, loss of p53 or ARF tumor suppressor activity may support rapid proliferation in cells with high NS (24). These studies suggest that the role and activity of NS in

Table 2. Results of univariate and multivariate analyses of disease-free and overall survival.

	Disease-free survival		Overall survival	
Univariate analysis	P-value		P-value	
Tumor dimension	**0.013**		**0.013**	
Extracapsular extension	**0.000**		**0.000**	
Grade	0.091		**0.043**	
Lymph node metastasis	**0.000**		**0.000**	
Lymphovascular invasion	**0.000**		**0.000**	
Perineural invasion	**0.000**		**0.000**	
T stage	**0.001**		**0.000**	
NS expression	**0.009**		**0.024**	
Multivariate analysis	P-value	95% confidence interval	P-value	95% confidence interval
Tumor dimension	0.620	0.628–2.184	0.283	0.764–2.505
Extracapsular extension	0.108	0.895–3.098	0.150	0.844–3.013
Grade	0.465	–	0.475	–
Lymph node metastasis	**0.005**	–	**0.001**	–
Lymphovascular invasion	0.163	0.766–4.890	0.263	0.670–4.316
Perineural invasion	**0.021**	1.240–14.055	**0.008**	1.530–17.202
T stage	0.459	–	0.534	–
NS expression	0.398	0.379–1.814	0.811	0.421–1.967

Bolded entries are statistically significant P-values.

tumor development may be affected by the molecular interaction between NS and other proteins. In the light of these studies, it is not unexpected that contradictory results emerge, as in our study.

Based on the results of our study, we conclude that NS is a favorable prognostic marker for gastric cancer patients. Our results are in conflict with prior studies. The prognostic effect of NS in gastric cancer remains to be solved. Further investigation of the functional interactions of NS in cell growth and proliferation will provide more insight into better understanding their roles in cancer development and prognosis. In the near future, prolonged survival due to the activation of NS may be an aspect of target-oriented treatment options for stomach cancer.

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