NPM1 is a prognostic biomarker and correlated with clinicopathological characteristics in gastric cancer

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Abstract

**Background and aims:** NPM1 plays an important role in the occurrence and development of leukemia, and its role in some solid tumors has gradually received attention. The purpose of this study is to explore the expression of NPM1 in gastric cancer (GC) tissues, to study the relationship between NPM1 expression and clinicopathological characteristics of GC patients, and to study the influence of NPM1 expression levels on the prognosis of GC patients.

**Methods:** The study was conducted from tissue samples obtained after radical gastrectomy in 106 patients with GC. We used tissue microarray immunohistochemical analysis to examine the expression level of NPM1 in different GC and adjacent tissues, and finally analyzed the relationship between NPM1 expression, clinicopathological factors and survival rate.

**Results:** Compared with the corresponding adjacent tissues, NPM1 is low-expressed in 75.5% of GC tissues. High NPM1 expression group was associated with better overall survival rate and disease free survival rate compared to low NPM1 expression group with GC ($p<0.01$).

**Conclusion:** The assessment of NPM1 expression in GC samples may represent a useful tool for GC diagnosis and prognostic prediction. Nonetheless, our study should be regarded as a preliminary study, and its predictive value as a single-center retrospective study is limited.

Introduction

Gastric cancer (GC) is the most common gastrointestinal tumor in the world and one of the main causes of cancer-related deaths[1, 2]. Early GC is asymptomatic or non-specific, and the diagnosis is often at an advanced stage, resulting in a low 5-year survival rate[3-6]. The incidence of GC has obvious regional characteristics, it is more likely to occur in East Asia and the mortality rate is high[7]. Personalized treatment for GC based on markers is considered as one of the methods to improve the 5-year overall survival (OS) rate of patients with GC. Finding new molecular biomarkers for GC is of great significance to improve the level of diagnosis and treatment[8, 9].

Nucleophosmin1 (NPM1, also known as B23) is a nucleolar shuttle phosphoprotein containing 294 amino acids[10]. It can quickly shuttle between the cytoplasm and the nucleus. This function determines that NPM1 can participate in many cells biological processes and perform different functions[11]. For example, it can be used as a molecular chaperone to regulate the function of histones[12]. It can also regulate the assembly of ribosomes[13, 14], DNA repair[15, 16] and cell apoptosis[17, 18] and so on. Over the years, numerous studies have shown that NPM1 can cause acute myeloid leukemia in the case of mutation or continuous high expression, and it plays a vital role in the occurrence and development of acute myeloid leukemia[19-21]. NPM1 can also participate in the occurrence and development of a variety of solid tumors at the same time[10, 22, 23].
So far, there are few studies on the relationship between NPM1 and GC, and controversy still exists between the research results. One study reported that expression level of NPM1 was significantly reduced in GC samples compared to matched non-tumor tissues samples and the low expression of NPM1 is obviously related to the distant metastasis of GC [24]. Another study reported that NPM1 expression significantly higher in GC tissues than in adjacent noncancerous tissues and the expression rates of NPM1 was significantly higher in patients with distant metastasis and more advanced tumor stages[25].

In view of the important role of NPM1 in neoplastic diseases and the recent contradictory research status in gastric cancer, it is necessary to do further research on its role and mechanism in gastric cancer. In this study, tissue microarray was used to analyzed NPM1 protein expression between GC tissues and matched noncancerous gastric samples. We also assessed the possible association between NPM1 and different clinicopathological features. Furthermore, we analyzed and evaluated the impact of NPM1 expression level on the prognosis of gastric cancer patients. Finally, a receiver operating characteristic (ROC) curve was generated to investigate the biomarker potential of NPM1 in GC prognostic diagnosis.

Methods

Patients

This study was approved by the research ethics committee at the Lanzhou University Second Hospital (NO:2021A-561). One hundred and six patients with primary GC that underwent surgical resection from Department of general surgery of Lanzhou University Second Hospital between January 2015 and October 2016 were adopted in this study. The recruited criteria: 1) A histological diagnosis of gastric adenocarcinoma. 2) The patients age ≥18 yeas. 3) Patients undergoing surgical resection of primary GC. 4) All data about pathological, treatment, surgical, and follow-up is complete. 5) The patient has signed an informed consent. The exclusion criteria: 1) Cases that died before discharge. 2) Receipt of preoperative chemoradiation or neoadjuvant chemotherapy, and 3) Multiple cancers patients within five years. Of the 106 patients in cohort, 79 had received standard adjuvant postoperative chemotherapy (5-fluorouracil or oxaliplatin-based regimen) within first month after surgery, and 27 patients had not due to the financial reasons. None of them accepted neoadjuvant chemotherapy or perioperative chemoradiation. Tumors were histologically staged according to the 7th edition of the TNM classification by the American Joint Committee on Cancer (AJCC). All patients participating in this research understand the content of this study and agree to participate in it.

Follow-up is conducted through normal outpatient clinics and telephone. The follow-up time is 2 weeks after discharge, once every 3 months in the first and second years, and 6 months in the following 3 years. The overall survival time is the time interval from surgery to the end of follow-up or death. Disease-free survival (DFS) time refers to the time interval between the first recurrence or the end of the follow-up during the postoperative follow-up period.

Tissue microarray construction
Tissue microarray was built from 106 patients with excised specimens of primary gastric tumors. The hematoxylin and eosin stained pathological sections of the included cases were read, and the H-E stained pathological sections and the representative positions on the corresponding wax blocks were marked. Use a puncher to punch holes on the sample wax block to obtain tissue columns, and load them in the chip wax block according to the sequence of arrangement. Each sample has 3 multiple points.

**Immunohistochemical staining**

Use immunohistochemical staining analysis according to the procedure previously described by the manufacturer[26]. For antigen retrieval, the TMA slides are dewaxed, rehydrated and boiled in a pressure pot with sodium citrate buffer (pH 6.0). The TMA slides were blocked with inhibitor (3% hydrogen peroxide) for thirty min at 37°C after antigen retrieval. Use NPM1 antibody dilution for immunohistochemical staining (NPM1,1:200, Abcom, Cambridge, USA). Use the primary antibody for 25 minutes at room temperature and overnight at 4°C. Then rewarmed for 15 minutes, wash with TBS 3 times, add 50μL of secondary antibody, and incubate for 25 minutes at room temperature. Rinse 3 times with TBS, DAB develops color, hematoxylin counterstain, rinse with water, dehydrate, and mount the film. Observe the expression of NPM1 protein under an optical microscope and take photos of the pictures.

**Evaluation of immunostaining**

Under the microscope, the complete tissue structure can be observed and the brown-yellow particles with obvious distribution in the background cells are judged as positive. The immunoreactive score (IRS) method[27] was used for semi-quantitative scoring according to the degree of staining: 0=no, 1=light yellow, 2= brown, and 3= dark brown. Then the percentage of positively stained tumor cells in each field were calculated, and take the average value for score: the percentage of positive cells no is 0, less than 10% is 1, and 10%-50% is 2, 51%-80% is 3, more than 80% is 4. The two scores are multiplied: 0 is considered negative (0+), 1 to 4 are considered weakly positive (1+), 5 to 8 are considered moderately positive (2+), and 9 to 12 are considered strong positive (3+). If there are multiple visual fields with different scores in the same specimen, the average value of the maximum value and the minimum value is taken as the immunohistochemical score. All arrays were reviewed by two unsuspecting pathologists. Review and discuss the inconsistent cases again until a consensus reached.

**Statistical analysis**

The relationship between the clinicopathological characteristics and expression level of NPM1 were assessed using the Pearson's chi-squared test ($\chi^2$ test) or Fisher's exact probability test. Kaplan-Meier method was used to build OS and DFS curves. The significance of OS and DFS between the NPM1 high and low expression groups was tested by log-rank test. Cox proportional hazards regression model was used for univariate and multivariate survival analysis. The ROC curve is designed to evaluate the
diagnostic value of NPM1 in GC. Each statistical test was two-sided, the difference is statistically significant when \( p<0.05 \). All statistical analyses were performed using SPSS 23.0 statistical software package (SPSS, Chicago, IL, USA).

**Results**

**The expression of NPM1 in gastric cancer and patient’s clinicopathological characteristics**

Representative expression levels (0+, 1+, 2+, 3+) of NPM1 is shown in Figure 1. The cohort consisted of 83 (78.3%) male and 23 (21.7%) female, with a median patient age of 64 (range 31–88) years. Sixty seven percent of tumors are located in the antrum of the stomach, 22.6% are located in the body of the stomach, and about 10.4% are located at the junction of the cardia and the esophagus. The intestinal type and diffuse type in Lauren classification accounted for 56.6% and 43.4 % respectively. Noticeably, 42 cases (39.6%) were in stages Ib-II, 64 cases (60.4%) were in stages III-IV (according to the 7th edition of the TNM classification by AJCC). Seventy-nine patients had received standard adjuvant postoperative chemotherapy (5-fluorouracil or oxaliplatin-based regimen) within first month after surgery, and 27 patients had not due to the financial reasons. The clinicopathologic data and demographic features of the 106 GC patients are summarized in Table 1.

**Relationship between clinicopathological factors and NPM1 expression**

Fifteen clinicopathological factors of high and low expression of NPM1 were compared separately (Table 2). There are significant differences in the venous invasion and AJCC stage (\( p<0.05 \)).

**Relationship between NPM1 expression level and prognosis of GC**

Kaplan-Meier curves for OS and DFS rates in NPM1 high and NPM1 low expression groups were shown in Figure 2. The OS rates in patients with high and low NPM1 expression were 49.3% and 33.1%, respectively (Figure 2A). Compared with the low NPM1 expression group, the high NPM1 expression group showed significantly better OS (by log-rank test \( p<0.05 \)). The DFS rates in patients with high and low NPM1 expression was 34.3% and 19.3%, respectively (Figure 2B). Compared with the low NPM1 expression group, the high NPM1 expression group showed significantly better DFS (by log-rank test \( p<0.01 \)).
Univariate and multivariate COX regression analyses of OS and DFS

In the univariate and multivariate COX regression analyses of OS, we analyzed: age, gender, location of tumor, tumor size, pT stage (pathological assessment of primary tumor), pN stage (pathological assessment of regional lymph nodes), AJCC stage, lymphatic invasion, Lauren's classification, venous invasion, carcino-embryonic antigen (CEA), carbohydrate antigen199 (CA199), carbohydrate antigen724 (CA724), α-fetoprotein (AFP), carbohydrate antigen125 (CA125), and NPM1 expression. In the univariate analyses, age, tumor size, pT stage, AJCC stage, Venous invasion and NPM1 expression were selected as significant factors for OS. Meanwhile, in the multivariate analyses, age, tumor size, pT stage, AJCC stage, Venous invasion and NPM1 expression were independent predictors of OS in curative gastrectomy patients (Table 3).

In the univariate and multivariate COX regression analyses of DFS, we analyzed: age, gender, location of tumor, tumor size, pT stage, pN stage, AJCC stage, lymphatic invasion, Lauren's classification, venous invasion, CEA, CA199, CA724, AFP, CA125, and NPM1 expression. In the univariate analyses, age, pT stage, pN stage, AJCC stage, Venous invasion and NPM1 expression were selected as significant factors for DFS. Meanwhile, in the multivariate analyses, age, pT stage, pN stage, AJCC stage, Venous invasion and NPM1 expression were independent predictors of DFS in curative gastrectomy patients (Table 4).

Diagnostic accuracy of NPM1 for predicting the prognosis of patients with GC

The ROC curve for diagnostic accuracy of NPM1 for the predicting the prognosis of patients with GC is presented in Figure 3. NPM1 scores yielded an area under the curve (AUC) of 0.712 (95% CI, 0.616-0.818), with a sensitivity of 77.3% and a specificity 78.2%, a cutoff value at 2.0 points.

Discussion

For a long time, numerous studies have shown that NPM1 can cause acute myeloid leukemia in the case of mutation or continuous high expression, and it plays a vital role in the occurrence, development and prognosis of acute myeloid leukemia[19-21]. NPM1 can also participate in the occurrence and development of a variety of solid tumors at the same time[10, 22, 23]. In oral squamous cell carcinoma, higher NPM1 expression is significantly associated with larger tumor size, lymph node metastasis, and advanced clinical stage[28]. Multivariate analysis results show that higher NPM1 is associated with a worse prognosis[28]. Another study shows that the expression level of NPM1 in lung adenocarcinoma samples was higher than that in adjacent normal paracancerous tissues. NPM1 has high specificity and
sensitivity for the diagnosis and prognosis assessment of lung adenocarcinoma[29]. Recently study reports that NPM1 expression is significantly increased in colorectal cancer and is associated with a poorer 5-year overall survival rate[30]. Above studies are examples of the high expression of NPM1 in different tumors. Its high expression promotes the biological behavior of the tumor and predicts a poor prognosis. However, it is interesting that there are different reports showing that NPM1 is lower-expressed in some tumors. Its low expression promotes the biological behavior of tumors and predicts a poor prognosis. Luo etc.[31] reported that NPM1 has a low expression in bladder cancer cells, which is also related to the poor prognosis of bladder cancer. Knockdown of NPM1 expression in bladder cancer cell lines can significantly improve tumor cell migration and invasion capabilities. The silencing of NPM1 will accelerate the tumorigenicity of drug-resistant bladder cancer cells. Karhemo etc.[32] reported that NPM1 is lower expressed in breast cancer, and the decrease of NPM1 protein level in breast cancer is related to poor prognosis. Histologically, the luminal epithelial cells of normal breasts showed high levels of NPM1 expression. The overexpression of NPM1 in breast cancer cells MDA-MB-231 stopped their growth in soft agar. NPM1 has a tumor suppressor effect in breast cancer. It can be seen that due to the different types of tumors, NPM1 plays different roles. It acts as a tumor-promoting factor in some tumors, but it acts as a tumor suppressor in other tumors.

So far, there are few studies on the relationship between NPM1 and GC, and controversy still exists between the research results. One study reported that expression level of NPM1 was significantly reduced in GC samples compared to matched non-tumor tissues samples and the low expression of NPM1 is obviously related to the distant metastasis of GC[24]. Another study reported that NPM1 expression significantly higher in GC tissues than in adjacent noncancerous tissues and the expression rate of NPM1 was significantly higher in patients with distant metastasis and more advanced tumor stages[25]. In view of the important role of NPM1 in neoplastic diseases and the recent contradictory research status in gastric cancer, it is necessary to do further research on its role in GC.

In this study, we used the tissue microarray method to detect the expression level of NPM1 in the GC tissues and adjacent normal tissues in 106 GC patients. The result of this study shows that in 75.5% of matched samples of gastric cancer and adjacent tissues, the NPM1 in gastric cancer samples was lower expressed than that of matched normal adjacent tissues. It can be seen that the expression level of NPM1 in most gastric cancer tissues is significantly lower than that of normal adjacent tissues.

Survival analysis results show that low expression of NPM1 was significantly associated with worse OS and DFS. The OS rate in patients with high and low NPM1 expression was 49.3% and 33.1%, respectively. Compared with the low NPM1 expression group, the high NPM1 expression group showed significantly better OS (by log-rank test \(p<0.05\)). The DFS rate in patients with high and low NPM1 expression was 34.3% and 19.3%, respectively. Compared with the low NPM1 expression group, the high NPM1 expression group showed significantly better DFS (by log-rank test \(p<0.01\)). It can be seen that the expression level of NPM1 has a direct impact on the OS and DFS in GC patients. NPM1 exists as a tumor suppressor in GC.
In the univariate COX regression analyses, age, tumor size, pT stage, AJCC stage, Venous invasion and NPM1 expression were selected as significant factors for OS. Meanwhile, in the multivariate COX regression analyses, age, tumor size, pT stage, AJCC stage, Venous invasion and NPM1 expression were independent predictors of OS in curative gastrectomy patients.

In the univariate COX regression analyses, age, pT stage, pN stage, AJCC stage, Venous invasion and NPM1 expression were selected as significant factors for DFS. Meanwhile, in the multivariate COX regression analyses, age, pT stage, pN stage, AJCC stage, Venous invasion and NPM1 expression were independent predictors of DFS in curative gastrectomy patients.

The ROC curve for diagnostic accuracy of NPM1 for the predicting the prognosis of patients with GC was demonstrated. NMP1 scores yielded an AUC of 0.712 (95% CI, 0.616-0.818), with a sensitivity of 77.3% and a specificity 78.2%, a cutoff value at 2.0 points. The assessment of NPM1 expression in GC samples may represent a useful tool for GC diagnosis and prognostic prediction.

Our research has some limitations. In the first place, although the number of GC patients in this study is relatively large (n=106), this is a single-center, retrospective study. In order to obtain a more reliable analysis of the clinical significance of NPM1 in GC, a multicenter study includes a larger number of patients is also needed. In the second place, we used immunostaining to examine the expression of NPM1 in the central and peripheral parts of each GC tissue; however, considering the heterogeneity, the expression level of NPM1 at the sampling site may not be representative of the entire tumor area.

**Conclusion**

The assessment of NPM1 expression in GC samples may represent a useful tool for GC diagnosis and prognostic prediction. Nonetheless, our study should be regarded as a preliminary study, and its predictive value as a single-center retrospective study is limited.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the research ethical review board at the Lanzhou University Second Hospital (NO:2021A-561). All experiments were performed in accordance with relevant guidelines and regulations. All participants in this study (or their parents/legal guardians/close relatives) obtained written informed consent to participate in the study.

**Consent for publication**

All authors have given their consent for publication in this journal.
Availability of data and materials

All data generated or analyzed during this study are included in this article. For further inquiries, please contact the corresponding author directly. There are no multimedia and supplementary materials. Each file not exceed 10 MB in size.

Competing interests

Authors declare that they have no conflict of interest.

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Author Contributions

CAG and HBL conceived and designed the study. XLS, THX, XMC, and SBY extracted and collected the data. XLS, AZ, and WAW analyzed the data. CAG, WJW, and XLS performed experiments. CAG, and AZ prepared figures and tables. CAG, XLS, and HBL wrote and revised the draft manuscript. All authors reviewed and approved the final manuscript.

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Tables

Table 1

Clinicopathological characteristics of GC patients.
| Variables                        | n=106 |
|----------------------------------|-------|
| Age, median (range)              | 64(31-88) |
| Gender                           |       |
| Male                             | 83(78.3%) |
| Female                           | 23(21.7%) |
| Tumor location                   |       |
| Antrum                           | 71(67.0%) |
| Body                             | 24(22.6%) |
| Cardia                           | 11(10.4%) |
| Tumor size                       |       |
| ≥5cm                             | 48(45.3%) |
| <5cm                             | 58(54.7%) |
| pT stage                         |       |
| T1-T3                            | 40(37.7%) |
| T4                               | 66(62.3%) |
| Lymph-node metastasis            |       |
| Positive                         | 86(81.1%) |
| Negative                         | 20(18.8%) |
| Lymphatic invasion               |       |
| Positive                         | 78(73.6%) |
| Negative                         | 28(26.4%) |
| pN stage                         |       |
| N0                               | 20(18.8%) |
| N1                               | 19(17.9%) |
| N2                               | 26(24.5%) |
| N3                               | 41(38.7%) |
| Lauren's classification          |       |
| Intestinal type                  | 60(56.6%) |
| Diffuse type                     | 46(43.4%) |
| Variables                     | n=106 |
|-------------------------------|-------|
| AJCC stage                    |       |
| Ib-II                         | 42(39.6%) |
| III-IV                        | 64(60.4%) |
| Venous invasion               |       |
| Positive                      | 73(68.9%) |
| Negative                      | 33(31.1%) |
| CEA                           |       |
| Positive                      | 58(55.0%) |
| Negative                      | 48(45.0%) |
| CA199                         |       |
| Positive                      | 44(41.5%) |
| Negative                      | 62(58.5%) |
| CA724                         |       |
| Positive                      | 52(49.1%) |
| Negative                      | 54(50.9%) |
| AFP                           |       |
| Positive                      | 11(10.4%) |
| Negative                      | 95(89.6%) |
| CA125                         |       |
| Positive                      | 22(20.8%) |
| Negative                      | 84(79.2%) |
| NPM1 expression               |       |
| Low                           | 80(75.5%) |
| High                          | 26(24.5%) |
| Adjuvant chemotherapy         |       |
| Yes                           | 79(74.5%) |
| No                            | 27(25.5%) |
Table 2

Comparison of clinicopathological parameters between NPM1 negative and positive gastric cancer patients.

| Variables                                                                 | n=106 |
|---------------------------------------------------------------------------|-------|
| Abbreviations: NPM1, nucleophosmin1. pT stage, pathological assessment of primary tumor. pN stage, pathological assessment of regional lymph nodes. CEA, carcino-embryonic antigen. CA199, carbohydrate antigen199. CA724, carbohydrate antigen 724. AFP, α-fetoprotein. CA125, carbohydrate antigen 125. |       |
|                      | Low (n=80) | High (n=26) | p     |
|----------------------|------------|-------------|-------|
| **NPM1 expression**  |            |             |       |
| **Age (years), median (range)** | 66 (32-88) | 65 (31-87) | 0.102 |
| **Gender**           |            |             |       |
| Male                 | 63 (78.8%) | 20 (76.9%)  | 0.158 |
| Female               | 17 (21.2%) | 6 (23.1%)   |       |
| **Tumor location**   |            |             |       |
| Antrum               | 56 (70.0%) | 15 (57.7%)  | 0.154 |
| Body                 | 16 (20.0%) | 8 (30.8%)   |       |
| Cardia               | 8 (10.0%)  | 3 (11.5%)   |       |
| **Tumor size**       |            |             |       |
| ≥5cm                 | 36 (45.0%) | 12 (46.2%)  | 0.149 |
| <5cm                 | 44 (55.0%) | 14 (53.8%)  |       |
| **Lauren's classification** |        |             |       |
| Intestinal type      | 46 (57.5%) | 14 (53.8%)  | 0.264 |
| Diffuse type         | 34 (42.5%) | 12 (46.2%)  |       |
| **pT stage**         |            |             |       |
| T1-T3                | 29 (36.3%) | 11 (42.3%)  | 0.056 |
| T4                   | 51 (63.7%) | 15 (57.7%)  |       |
| **pN stage**         |            |             |       |
| N0                   | 15 (18.8%) | 5 (19.2%)   | 0.325 |
| N1                   | 14 (17.5%) | 5 (19.2%)   |       |
| N2                   | 20 (25.0%) | 6 (23.1%)   |       |
| N3                   | 31 (38.8%) | 10 (38.5%)  |       |
| **AJCC stage**       |            |             |       |
| Ib-II                | 26 (32.5%) | 16 (61.5%)  | 0.010 |
| III-IV               | 54 (67.5%) | 10 (38.5%)  |       |
|                     | NPM1 expression |          |          |  |
|---------------------|-----------------|----------|----------|--|
|                     | Low(n=80)       | High(n=26)|          |  |
| Venous invasion     |                 |          |          |  |
| Positive            | 64(80.0%)       | 9(34.6%) | 0.023    |  |
| Negative            | 16(20.0%)       | 17(65.4%)|          |  |
| Lymphatic invasion  |                 |          |          |  |
| Positive            | 60(75.0%)       | 18(69.2%)| 0.259    |  |
| Negative            | 20(25.0%)       | 8(30.8%) |          |  |
| CEA                 |                 |          |          |  |
| Positive            | 42(52.5%)       | 16(61.5%)| 0.092    |  |
| Negative            | 38(47.5%)       | 10(38.5%)|          |  |
| CA199               |                 |          |          |  |
| Positive            | 33(41.3%)       | 11(42.3%)| 0.328    |  |
| Negative            | 47(58.7%)       | 15(57.7%)|          |  |
| CA724               |                 |          |          |  |
| Positive            | 40(50.0%)       | 12(46.2%)| 0.219    |  |
| Negative            | 40(50.0%)       | 14(53.8%)|          |  |
| AFP                 |                 |          |          |  |
| Positive            | 8(10.0%)        | 3(11.5%) | 0.146    |  |
| Negative            | 72(90.0%)       | 23(88.5%)|          |  |
| CA125               |                 |          |          |  |
| Positive            | 16(20.0%)       | 6(23.1%) | 0.287    |  |
| Negative            | 64(80.0%)       | 20(76.9%)|          |  |

Abbreviations: NPM1, nucleophosmin1. pT stage, pathological assessment of primary tumor. pN stage, pathological assessment of regional lymph nodes. CEA, carcino-embryonic antigen. CA199, carbohydrate antigen199. CA724, carbohydrate antigen 724. AFP, α-fetoprotein. CA125, carbohydrate antigen 125.

Table 3

Univariate and multivariate COX regression analyses of OS predictors in patients with GC (n=106).
| Variable                  | Univariate COX regression | Multivariate COX regression |
|---------------------------|---------------------------|----------------------------|
|                           | N  | HR   | 95% CI  | p-value  | HR | 95% CI   | p-value |
| Age (years)               |    |      |         |          |    |          |         |
| <70                       | 33 | 2.369| 1.733-3.046| 0.015    |
| ≥70                       | 73 | 2.254| 1.856-2.965| 0.025    |
| Gender                    |    |      |         |          |    |          |         |
| Male                      | 83 | 0.761| 0.463-1.214| 0.254    |
| Female                    | 23 | 1.062| 0.743-1.325|          |
| Location of tumor         |    |      |         |          |    |          |         |
| Antrum                    | 71 | 1.062| 0.743-1.325| 0.124    |
| Non-antrum                | 35 | 1.938| 1.249-2.367|          |
| Tumor size                |    |      |         |          |    |          |         |
| ≥5cm                      | 48 | 2.354| 1.698-3.021| 0.002    |
| <5cm                      | 58 | 1.938| 1.249-2.367| 0.003    |
| pT stage                  |    |      |         |          |    |          |         |
| T1-T3                     | 40 | 1.785| 1.245-2.021| 0.023    |
| T4                        | 66 | 1.865| 1.256-2.214| 0.005    |
| pN stage                  |    |      |         |          |    |          |         |
| N0                        | 20 | 1.564| 1.021-1.986| 0.156    |
| N1-N3                     | 86 | 1.564| 1.021-1.986|          |
| AJCC stage                |    |      |         |          |    |          |         |
| Ib-II                     | 42 | 2.325| 1.654-3.021| 0.029    |
| III-IV                    | 64 | 2.158| 1.433-2.986| 0.032    |
| Lymphatic invasion        |    |      |         |          |    |          |         |
| Positive                  | 78 | 1.445| 1.015-1.965| 0.452    |
| Negative                  | 28 | 2.354| 1.698-3.021|          |
| Variable                        | Univariate COX regression |                |               | Multivariate COX regression |                |               |
|--------------------------------|---------------------------|----------------|---------------|-----------------------------|----------------|---------------|
|                                | N  | HR      | 95% CI        | $$p$$-value    | HR      | 95% CI        | $$p$$-value |
| Lauren's classification        | 60 | 0.991   |               | 0.991          | 1.00    | 1.00-1.00     | 0.714       |
| Intestinal type                | 46 | 1.251   | 0.856-1.834   | 0.091          | 1.935   | 1.003-2.465   | 0.012       |
| Diffuse type                   | 46 | 1.251   | 0.856-1.834   | 0.091          | 1.935   | 1.003-2.465   | 0.012       |
| Venous invasion                |    | 0.048   |                | 0.012          | 0.012   |                |             |
| Positive                       | 73 |         |                |               | 1.935   | 1.003-2.465   |             |
| Negative                       | 33 | 1.785   | 1.124-2.215   |               | 1.935   | 1.003-2.465   |             |
| CEA                            |    | 0.069   |                |               | 1.935   | 1.003-2.465   |             |
| Positive                       | 58 |         |                |               | 1.935   | 1.003-2.465   |             |
| Negative                       | 48 | 1.255   | 0.645-1.855   |               | 1.935   | 1.003-2.465   |             |
| CA199                          |    | 0.122   |                |               | 1.935   | 1.003-2.465   |             |
| Positive                       | 44 |         |                |               | 1.935   | 1.003-2.465   |             |
| Negative                       | 62 | 0.966   | 0.542-1.455   |               | 1.935   | 1.003-2.465   |             |
| CA724                          |    | 0.135   |                |               | 1.935   | 1.003-2.465   |             |
| Positive                       | 52 |         |                |               | 1.935   | 1.003-2.465   |             |
| Negative                       | 54 | 1.548   | 0.965-2.122   |               | 1.935   | 1.003-2.465   |             |
| AFP                            |    | 0.253   |                |               | 1.935   | 1.003-2.465   |             |
| Positive                       | 11 |         |                |               | 1.935   | 1.003-2.465   |             |
| Negative                       | 95 | 1.223   | 0.554-1.869   |               | 1.935   | 1.003-2.465   |             |
| CA125                          |    | 0.114   |                |               | 1.935   | 1.003-2.465   |             |
| Positive                       | 22 |         |                |               | 1.935   | 1.003-2.465   |             |
| Negative                       | 84 | 0.965   | 0.258-1.562   |               | 1.935   | 1.003-2.465   |             |
| NPM1 expression                |    | 0.015   |                | 0.032          | 0.015   |                |             |
| Low                            | 80 |         |                |               | 1.875   | 1.324-2.433   |             |
| High                           | 26 | 1.965   | 1.354-2.432   |               | 1.875   | 1.324-2.433   |             |
## Abbreviations: NPM1, nucleophosmin1. pT stage, pathological assessment of primary tumor. pN stage, pathological assessment of regional lymph nodes. CEA, carcino-embryonic antigen. CA199, carbohydrate antigen 199. CA724, carbohydrate antigen 724. AFP, α-fetoprotein. CA125, carbohydrate antigen 125. N, Number of patients. HR, Hazard ratio. CI, Confidence interval.

| Variable | Univariate COX regression | Multivariate COX regression |
|----------|---------------------------|----------------------------|
|          | N  | HR  | 95% CI | p-value | HR  | 95% CI | p-value |

Table 4

Univariate and multivariate COX regression analyses of DFS predictors in patients with GC (n=106).
| Variable                      | Univariate COX regression | Multivariate COX regression |
|-------------------------------|---------------------------|-----------------------------|
|                               | N  | HR  | 95% CI       | p-value | HR  | 95% CI       | p-value |
| Age (years)                  |    |     |               |         |     |               |         |
| <70                           | 33 |     |               |         |     |               |         |
| ≥70                           | 73 | 2.448| 2.021-3.147  | 0.023   | 2.543| 2.035-3.011  | 0.036   |
| Gender                       |    |     |               | 0.325   |     |               |         |
| Male                          | 83 |     |               |         |     |               |         |
| Female                        | 23 | 0.865| 0.395-1.441  |         |     |               |         |
| Location of tumor             |    |     |               | 0.212   |     |               |         |
| Antrum                       | 71 |     |               |         |     |               |         |
| Non-antrum                   | 35 | 1.124| 0.632-1.624  |         |     |               |         |
| Tumor size                   |    |     |               | 0.062   |     |               | 0.089   |
| ≥5cm                         | 48 |     |               |         |     |               |         |
| <5cm                         | 58 | 1.533| 0.744-2.125  |         |     |               |         |
| pT stage                     |    |     |               | 0.012   |     |               | 0.011   |
| T1-T3                        | 40 |     |               |         |     |               |         |
| T4                            | 66 | 1.663| 1.114-2.142  |         |     |               |         |
| pN stage                     |    |     |               | 0.014   |     |               | 0.026   |
| N0                            | 20 |     |               |         |     |               |         |
| N1-N3                        | 86 | 1.978| 1.154-2.534  |         |     |               |         |
| AJCC stage                   |    |     |               | 0.035   |     |               | 0.022   |
| Ib-II                        | 42 |     |               |         |     |               |         |
| III-IV                       | 64 | 2.547| 1.553-3.154  |         |     |               |         |
| Lymphatic invasion           |    |     |               | 0.325   |     |               |         |
| Positive                     | 78 | 1.665| 1.125-2.012  |         |     |               |         |
| Negative                     | 28 |     |               |         |     |               |         |
| Variable                  | Univariate COX regression |           | Multivariate COX regression |           |
|--------------------------|---------------------------|-----------|----------------------------|-----------|
|                          | N | HR      | 95% CI     | p-value | HR   | 95% CI     | p-value |
| Lauren's classification  |   |         |            |         | 0.069 |           |         |
| Intestinal type          |   | 60       | 1.123      | 0.733-1.654 | 1.754 | 1.102-2.214 | 0.021 |
| Diffuse type             |   | 46       | 1.123      | 0.733-1.654 |       |           |         |
| Venous invasion          |   | 0.032    | 0.032      | 0.021    |       |           |         |
| Positive                 |   | 73       |            |          | 1.754 | 1.102-2.214 | 0.021 |
| Negative                 |   | 33       | 1.865      | 1.235-2.354 |       |           |         |
| CEA                      |   | 0.126    | 0.126      |          |       |           |         |
| Positive                 |   | 58       |            |          |       |           |         |
| Negative                 |   | 48       | 1.356      | 0.725-1.965 |       |           |         |
| CA199                    |   | 0.232    | 0.232      |          |       |           |         |
| Positive                 |   | 44       |            |          |       |           |         |
| Negative                 |   | 62       | 1.133      | 0.423-1.654 |       |           |         |
| CA724                    |   | 0.253    | 0.253      |          |       |           |         |
| Positive                 |   | 52       |            |          |       |           |         |
| Negative                 |   | 54       | 1.446      | 1.325-1.968 |       |           |         |
| AFP                      |   | 0.154    | 0.154      |          |       |           |         |
| Positive                 |   | 11       |            |          |       |           |         |
| Negative                 |   | 95       | 1.354      | 0.785-1.954 |       |           |         |
| CA125                    |   | 0.091    | 0.091      |          |       |           |         |
| Positive                 |   | 22       |            |          |       |           |         |
| Negative                 |   | 84       | 1.032      | 0.368-1.625 |       |           |         |
| NPM1 expression          |   | 0.023    | 0.023      | 0.011    |       |           |         |
| Low                      |   | 80       |            |          |       |           |         |
| High                     |   | 26       | 2.368      | 1.663-2.996 | 1.889 | 1.228-2.521 | 0.011 |
| Variable | Univariate COX regression | Multivariate COX regression |
|----------|---------------------------|----------------------------|
|          | N | HR | 95% CI | p-value | HR | 95% CI | p-value |

Abbreviations: NPM1, nucleophosmin1. pT stage, pathological assessment of primary tumor. pN stage, pathological assessment of regional lymph nodes. CEA, carcino-embryonic antigen. CA199, carbohydrate antigen199. CA724, carbohydrate antigen 724. AFP, α-fetoprotein. CA125, carbohydrate antigen 125. N, Number of patients. HR, Hazard ratio. CI, Confidence interval.

**Figures**
Figure 1

Representative score of the immunohistochemical staining of NPM1. Expression of NPM1 was observed in the nuclei of gastric cancer cells. (200× magnification).
Kaplan-Meier curves for OS and DFS rates in NPM1 low and NPM1 high expression groups. The OS rates in patients with high and low NPM1 expression were 49.3% and 33.1%, respectively (Figure 2A). Compared with the low NPM1 expression group, the high NPM1 expression group showed significantly better OS (by log-rank test \( p < 0.05 \)). The DFS rates in patients with high and low NPM1 expression were 34.3% and 19.3%, respectively (Figure 2B). Compared with the low NPM1 expression group, the high NPM1 expression group showed significantly better DFS (by log-rank test \( p < 0.01 \)).
Figure 3

Receiver operating characteristic (ROC) curve of NPM1 expression in GC samples for GC diagnosis and prognostic prediction.