Downregulation of NPM expression by Her-2 reduces resistance of gastric cancer to oxaliplatin

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Abstract. Nucleophosmin (NPM) and human epidermal growth factor receptor-2 (Her-2) are abnormally expressed in various types of human malignant tumors, including gastric cancer, and have been closely associated with cancer chemo-resistance. However, their interaction and roles in oxaliplatin resistance are not fully understood. Therefore, the present study aimed to elucidate the relationship between NPM and Her-2 in gastric cancer cell lines and clinical samples, and further investigated their role in the resistance of gastric cancer to oxaliplatin. Western blotting and reverse transcription-quantitative polymerase chain reaction confirmed that NPM and Her-2 expression were significantly upregulated in gastric cancer cells and clinical samples, and that their expression levels were strongly correlated. However, Her-2 expression was not affected by upregulation or downregulation of NPM expression in gastric cancer cells. Cell counting kit-8 assays demonstrated that the cell sensitivity to oxaliplatin decreased simultaneously with an increase in NPM expression. Furthermore, inhibition of Her-2 expression using trastuzumab significantly increased the sensitivity of the cells to oxaliplatin, which occurred simultaneously with the downregulation of NPM. These results indicated that inhibition of NPM, as a Her-2 downstream signal, may be a novel strategy to overcome oxaliplatin-resistant gastric cancer, and that trastuzumab and oxaliplatin may exhibit a synergistic antitumor effect in Her-2-positive gastric cancer cells.

Introduction

Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-associated mortality worldwide (1). According to GLOBOCAN 2012, ~1 million new gastric cancer cases, with an ~72% mortality rate, were diagnosed in 2012, of which ~50% occurred in Eastern Asia (predominantly in China) (2). The majority of patients present with an advanced stage of the disease at the time of diagnosis and, for them, palliative chemotherapy is the main choice of treatment (3). Oxaliplatin, which is a third-generation platinum analog, is widely used as a first-line chemotherapeutic agent for advanced gastric cancer (4). However, many patients eventually develop resistance after several courses of oxaliplatin-based chemotherapy, although the mechanisms remain poorly understood. Therefore, identification of suitable factors for predicting chemosensitivity or reducing resistance to oxaliplatin may be important for improving the therapeutic effect of oxaliplatin.

Nucleophosmin (NPM), which is also known as B23, numatrin or NO38, is a ubiquitously expressed nucleolar phosphoprotein that shuttles between the nucleus and cytoplasm (5). As a molecular chaperone, it is indispensable for various cellular processes, including ribosome biogenesis, centrosome duplication, DNA repair and stress responses (6). NPM is capable of binding to numerous partners in distinct cellular compartments and functions to maintain the dynamic balance between proliferation and apoptosis (7). Therefore, dysregulation of NPM expression and/or localization may contribute to tumorigenesis through various mechanisms. Previous studies have reported that NPM is highly expressed in proliferating cells and is involved in the tumorigenesis of hepatic (8), thyroid (9), gastric (10) and bladder (11) cancers. In addition, its upregulation in tumors has been correlated with a poor outcome (12). NPM is redistributed from the nucleus in response to cytotoxic drugs and genotoxic stress caused by inhibition of RNA polymerase I, exposure to DNA intercalating agents and UV damage (13,14). Furthermore, the upregulation of NPM in breast cancer cell lines was shown

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to promote resistance to estrogen (15). Conversely, silencing of NPM expression sensitized hepatoma cells to doxorubicin, cisplatin, lapatinib, sorafenib and UV-B (16); thus suggesting that the inhibition of NPM may be considered a novel therapeutic strategy for cancer treatment.

Human epidermal growth factor receptor-2 (Her-2) is a member of the epidermal growth factor receptor family of receptor tyrosine kinases, which have vital roles in the development and progression of various solid tumors, including gastric cancer (17). Since the downregulation of Her-2 was shown to promote the apoptosis of tumor cells (18), Her-2 is regarded as a potential target in breast and gastric cancer therapy. It has been suggested that malignant tumors with high expression levels of Her-2 may be more aggressive and more resistant to chemotherapy (19). The Her-2-specific antibody, trastuzumab (also known as Herceptin), is currently approved for the treatment of Her-2-positive breast cancer and metastatic gastric cancer as a result of the Trastuzumab for Gastric Cancer (ToGA) trial (20,21). As NPM and Her-2 have been shown to have overlapping roles in cell proliferation, apoptosis and gastric cancer development, and are considered therapeutic targets (22), it can be hypothesized that Her-2 and NPM expression may be correlated in the development of gastric cancer. The relationship and effect of NPM and Her-2 in chemoresistance require further elucidation.

The present study aimed to demonstrate the correlation between NPM and Her-2 expression in gastric cancer tissues and cell lines, as well as their role in the response of gastric cancer to oxaliplatin. In addition, the efficiency of using trastuzumab in combination with oxaliplatin for the treatment of gastric cancer was investigated, in order to provide a novel chemotherapeutic strategy for gastric cancer patients in clinical practice.

Materials and methods

Patients and clinical samples. Primary tumor tissues were obtained from 133 patients with gastric cancer who underwent surgical resection at the Department of General surgery, the Affiliated Hospital of Qingdao University (Qingdao, China) between January 2007 and November 2014. Patients enrolled in the study included 55 females and 78 males with an age range of 33-78 years (mean age, 61.4 years). No patients received chemotherapy or radiotherapy prior to surgery. In addition, 68 paracancer tissues that were located >5 cm from the edge of tumor were obtained. All samples were fixed in formaldehyde for 24 h, and then embedded in paraffin. All tumor tissue samples were confirmed to be adenocarcinoma histologically and the pathological diagnoses and stages were defined according to the International Union against Cancer tumor-node-metastasis (TNM) classification of malignant tumors (23). The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. Informed consent was obtained from all patients.

Immunohistochemistry (IHC). IHC was performed to detect the expression of NPM and Her-2 in the gastric cancer and paired paracancer tissues. The paraffin-embedded tissues were cut into 5-μm sections, deparaffinized and hydrated in graded ethanol. For antigen retrieval, the sections were incubated

in citrate buffer solution and heated in a microwave oven (pH 6.0) for 20 min. Endogenous peroxidase activity was inactivated by treating the sections with 0.3% hydrogen peroxide in methanol for 15 min. After washing in PBS, the sections were incubated with the primary antibodies against NPM (dilution 1:100; catalog no., ab180607; Abcam, Cambridge, MA, USA) and Her-2 (dilution 1:200; catalog no., ab178176; Abcam) overnight at 4°C. Antibody staining was detected using the avidin-biotin-peroxidase complex (PV9005; Zhongshan Jinqiao Biological Technology Co., Ltd., Beijing, China). Finally, sections were incubated in 3,3'-diaminobenzidine tetrahydrochloride for 3-10 min and counterstained with 0.1% hematoxylin. IHC staining was evaluated by two independent pathologists blinded to the clinicopathological characteristics according to the semiquantitative immunoreactivity score system, which involved measuring the intensity of staining (score 0-3) and the extent of staining (score 0, 0%; score 1, 0-10%; score 2, 10-50%; score 3, 50-100%). The final weighted score (0-9) for each sample, which was obtained by multiplying the scores for the intensity and extent of staining, was divided into two groups: The positive group with scores of 4-9 and the negative group with scores of 0-3.

Cell lines and cell culture. A total of four gastric cancer cell lines (SGC-7901, AGS, NCI-N87 and MKN-28) and two normal gastric epithelium cell lines (GES-1 and HFE-145), which show different expression levels of NPM and Her-2, were obtained from the Center for Medical Research of The Affiliated Hospital of Qingdao University (Qingdao, China). All cell lines were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China) and 1% penicillin/streptomycin at 37°C in 5% CO_2.

Plasmid and transfection. To explore the relationship between NPM and Her-2, the expression of Her-2 or NPM in the cell lines was altered using the human Her-2 expression vector (pcDNA3.1-Her-2), Her-2-small interfering (si) RNA, the human NPM expression vector (pcDNA3.1-NPM) or NPM-siRNA. Briefly, cells were transfected in six-well plates (5x10^5 cells/well) with pcDNA3.1-control, control siRNA, pcDNA3.1-Her-2, Her-2-siRNA, pcDNA3.1-NPM or NPM-siRNA using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Following transfection, the cells were incubated for 48-72 h in their normal media and the transfection efficiency was confirmed under microscope and by western blot analysis. Furthermore, following NPM transfection, the NPM overexpressing cells (AGSox) were incubated with oxaliplatin (Sigma-Aldrich; EMD Millipore, Billerica, MA, USA) alone (2.4 μg/ml) or in combination with 5 μg/ml trastuzumab (a monoclonal antibody that interferes with the Her-2 receptor) for 24 h and subsequently were used for oxaliplatin resistance analysis.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from the four gastric cancer cells and the two normal gastric epithelium cells using TRIzol reagent (Invitrogen;

Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol, and then quantification and purity assessment (A260/A280≥1.8) were performed using a spectrophotometer. RNA was reverse transcribed into cDNA using reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.), after which qPCR was performed on the LightCycler 480 Instrument II (Roche Diagnostics, Basel, Switzerland) using SYBR Premix Ex Taq reagents from the TaKaRa RNA PCR kit (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. The primer sequences for PCR amplification were as follows: Her-2 (230 bp) sense, 5'-TCTGCTGGAGGAATTGGTGT-3' and antisense, 5'-GACGCTTGTTCCCACCTTGA-3'; NPM (310 bp) sense, 5'-UGUCUGUACAGCCAACGGUTT-3' and antisense, 5'-ACCGUUGGCUGUACAGACATT-3'; and β-actin (135 bp) sense, 5'-ACCACAGTCCTGCATGCCAC-3' and antisense, 5'-TCCACCACCCTGTTGCTGTA-3'. The PCR thermal cycling conditions were as follows: One cycle at 95°C for 10 sec, followed by 40 cycles at 95°C for 5 sec and 60°C for 20 sec. Fold-changes in the mRNA expression levels of Her-2 and NPM were calculated following normalization to β-actin using the $2^{-\Delta\Delta Cq}$ method (24). To ensure experimental accuracy, all reactions were performed in triplicate. An RT-mius control was performed using ultrapure water. Normal tissue served as a negative control.

Western blot analysis. Cells were collected and lysed using radioimmunoprecipitation assay lysis buffer containing 0.01% phenylmethylsulfonyl fluoride, 150 nmol/l Tris (pH 8), 0.1% SDS, 0.2% EDTA, 1% Triton X-100 and 1% sodium deoxycholate, and supplemented with protease inhibitor and phosphatase inhibitor cocktails (Roche Diagnostics). The protein concentrations of the cellular extracts were determined using the bicinchoninic acid protein assay (Nanjing KeyGen Biotech, Co., Ltd., Nanjing, China), according to the manufacturer's protocol. Equal quantities (30-50 µg) of proteins were loaded onto an 8-12% SDS-polyacrylamide separating gel and transferred onto a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). The membrane was blocked with 1% bovine serum albumin (Shanghai Qcbio Science & Technologies Co., Ltd., Shanghai, China) in TBST and then incubated at 4°C overnight with anti-NPM (dilution 1:1,000; catalog no., 20314263-2; Bioworld Technology, Inc., St. Louis Park, MN, USA), anti-Her-2 (dilution 1:2,000; catalog no., ab178176; Abcam) and anti-GAPDH (dilution 1:2,000; catalog no., CW0100M; Beijing ComWin Biotech Co., Ltd. Beijing, China) rabbit polyclonal antibodies, followed by appropriate horseradish peroxidase-conjugated secondary antibodies (dilution, 1:500; catalog. no., SH026; Applied Biological Materials, Inc., Richmond, BC, Canada). Antibody staining was visualized by enhanced chemiluminescence (EMD Millipore) and the images were analyzed using Quantity One 4.31 software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Cell counting kit (CCK)-8 assay. The sensitivity of the gastric cancer cells with different expression levels of NPM to oxaliplatin were detected using the CCK-8 assay kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Briefly, cells were seeded into 96-well culture plates (10,000 cells/well) in 100 µl fresh medium containing various concentrations of oxaliplatin (0.3, 0.6, 1.2, 2.4, 4.8, 9.6 or 19.2 µg/ml). After a

Table I. Differential expression of Her-2 and NPM in gastric cancer tissues and non-cancerous tissues.

Tissue	NPM		Her-2	
	+	-	+	-
Gastric cancer	89 (66.9)	44 (33.1)	21 (15.8)	112 (84.2)
Paracancer	12 (17.7)	56 (82.4)	4 (5.9)	64 (94.1)
χ^2	43.69		4.05	
P-value	<0.0001		<0.05	

Data are presented as n (%). NPM, nucleophosmin; Her-2, human epidermal growth factor receptor-2.

Table II. Correlation between the expression of NPM and Her-2 in gastric cancer tissues only.

Variable	Her-2(+)	Her-2(-)	P-value
NPM(+)	24 (96.0)	75 (43.1)	<0.0001
NPM(-)	1 (4.0)	99 (56.9)	

Data are presented as n (%). NPM, nucleophosmin; Her-2, human epidermal growth factor receptor-2.

24-h incubation, 10 µl CCK-8 reagent was added to each well at 37°C for 4 h. The microplate reader (Bio-Rad Model 550; Bio-Rad Laboratories, Inc.) was used to determine the optical density (OD) of each well at 450 nm. CCK-8 assays were repeated three times on different days to verify the accuracy. The cell inhibition rate was calculated using the following formula: Cell inhibition rate (%)=[1 - (OD experiment - OD blank) / (OD control - OD blank)] x 100. The half maximal inhibitory concentration (IC_{50}) of oxaliplatin was obtained by interpretation of the graph.

Statistical analysis. Quantitative data are expressed as the mean ± standard deviation. Statistical comparisons were performed by one-way analysis of variance and χ^2 tests as appropriate. The SAS 9.0 software (SAS Inc., Chicago, IL, USA) was used for statistical analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of Her-2 and NPM in gastric cancer tissues. Expression of NPM and Her-2 in gastric cancer tissues was measured by semiquantitative IHC analyses (Fig. 1). NPM was localized predominantly in the nucleoli, nuclei and cytoplasm and showed different intensities and extents of staining; the positive expression rates in the cancer tissues and paracancer tissues were 66.9 and 17.7%, respectively. Conversely, Her-2 was expressed mainly in the membrane or cytoplasm of tumor epithelial cells, and the positive expression rates in the cancer tissues and paracancer tissues were 15.8 and 5.9%, respectively (Table I). These results showed that the expression levels
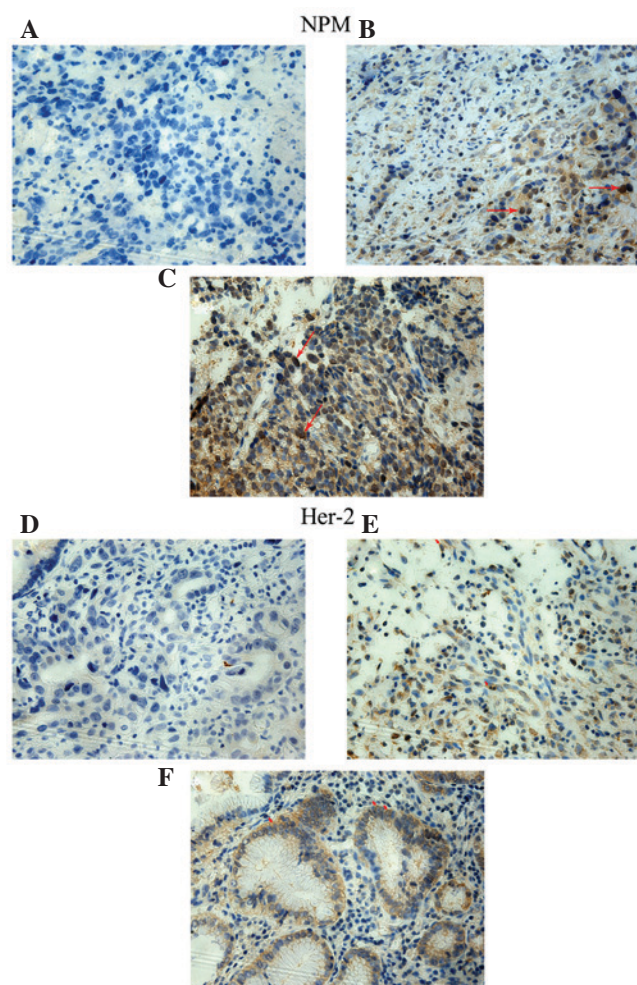
Figure 1. Expression of Her-2 and NPM in gastric cancer tissues. (A-C) NPM localized predominantly to the nucleoli, nuclei and cytoplasm of gastric cancer cells to varying intensities and degrees (indicated by long red arrows). (A) Negative expression of NPM. (B) Low intensity and percentage of NPM positive expression. (C) High intensity and percentage of NPM positive expression. (D-F) Her-2 was expressed predominantly in the membrane or cytoplasm of tumor epithelial cells (indicated by short red arrows). (D) Negative expression of Her-2. (E) Low intensity and percentage of Her-2 positive expression. (F) High intensity and percentage of Her-2 positive expression. Magnification, x200. NPM, nucleophosmin; Her-2, human epidermal growth factor receptor-2.
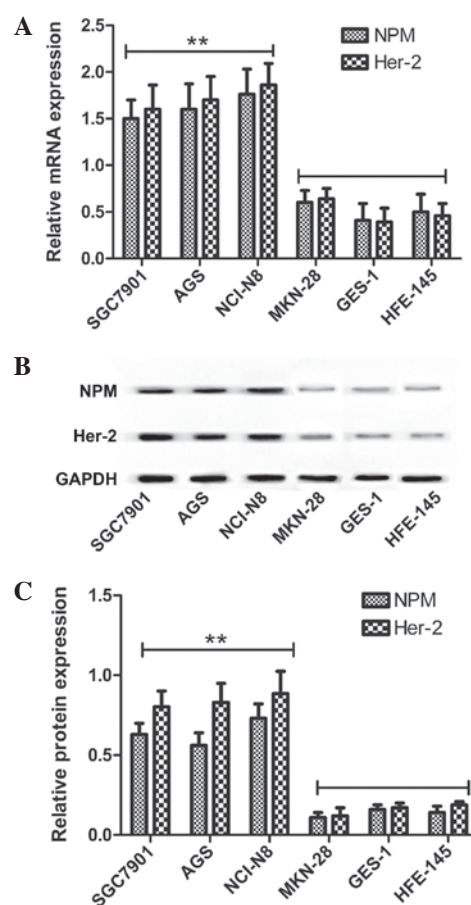

Figure 2. Expression of NPM and Her-2 in different gastric cell lines. (A) NPM and Her-2 mRNA expression levels in different gastric cancer or epithelium cell lines were determined by reverse transcription-quantitative polymerase chain reaction. (B) Protein expression of NPM and Her-2 in gastric cells was determined by western blotting. (C) Relative protein expression levels of NPM and Her-2 in different gastric cells were quantified by densitometry. **P<0.01. NPM, nucleophosmin; Her-2, human epidermal growth factor receptor-2.

of NPM and Her-2 were significantly higher in gastric cancer tissues than in the paracancer tissues (P<0.01 and P<0.05, respectively). In addition, 24 (96%) gastric carcinoma tissues exhibited positive Her-2 and NPM expression, and it was demonstrated there was a significant and positive correlation between NPM and Her-2 expression (P<0.0001; Table II).

NPM and Her-2 are overexpressed in gastric cancer cell lines. In our previous study, it was observed that the Her-2 expression level altered in accordance with the NPM expression level following NPM gene transfection (22), which suggested that there is a relationship between NPM and Her-2 in gastric cancer. In the present study, the expression levels of NPM and Her-2 were detected in four gastric cancer cell lines and two normal gastric epithelium cell lines using RT-qPCR and western blot analysis. The expression of the NPM protein, as detected using an NPM-specific antibody, correlated with

that of NPM mRNA in gastric cancer cell lines (Fig. 2). As compared with the normal gastric epithelium cell lines, NPM was significantly overexpressed in three of the four gastric cancer cell lines (Fig. 2A), suggesting that NPM may be considered a target for inhibition of gastric cancer cell activation. Furthermore, high mRNA and protein expression levels of Her-2 were detected in SGC7901, AGS and NCI-N87 cells, whereas both normal gastric epithelium cell lines exhibited low expression levels of Her-2 (Fig. 2B and C). The differences in the mRNA and protein expression levels of NPM and Her-2 between the malignant and benign gastric cells were significant, with the exception of the MKN-28 cell line.

Her-2 regulates NPM expression in gastric cancer cell lines. When further analyzing the relationship between NPM and HER-2 expression, it was demonstrated that the three gastric cancer cell lines that exhibited NPM overexpression were all positive for Her-2 expression, while the Her-2-negative gastric cancer cell line, MKN-28, also expressed low levels of NPM (Fig. 2). Altogether, all four gastric cancer cell lines exhibited a positive correlation between the expression levels of NPM and Her-2. In order to explore the interaction relationship
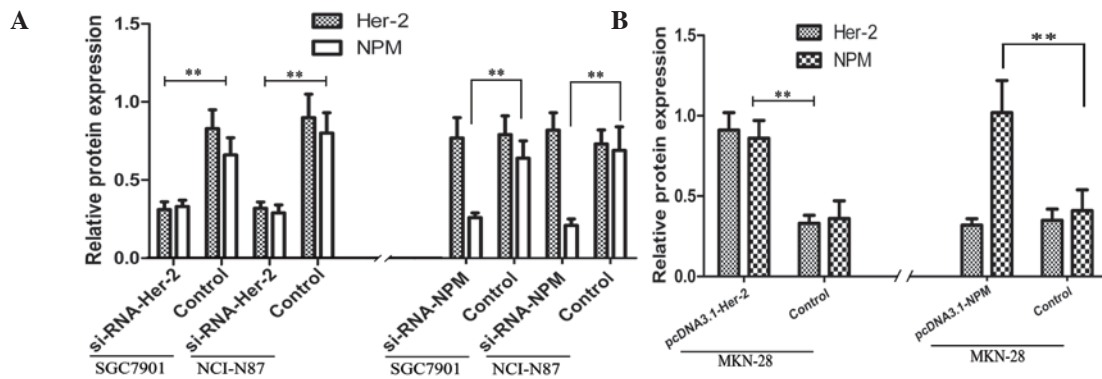
Figure 3. Her-2 regulates NPM expression in gastric cancer cell lines. (A) Downregulating Her-2 expression in gastric cancer cells using Her-2-specific siRNA altered NPM expression concordantly, while downregulating NPM expression using NPM-specific siRNA did not significantly alter Her-2 expression. (B) Upregulating Her-2 expression in MKN-28 gastric cancer cells using a Her-2-expressing plasmid altered NPM expression, while upregulating NPM expression in the same cells did not lead to a prominent change in Her-2 expression. **$P<0.01$. NPM, nucleophosmin; Her-2, human epidermal growth factor receptor-2; siRNA, small interfering RNA.
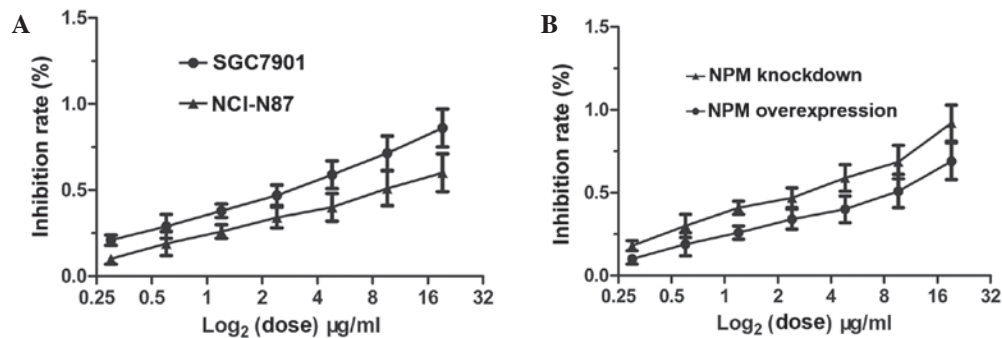

Figure 4. NPM overexpression correlates to resistance of gastric cancer cells to oxaliplatin, as determined using cell counting kit-8 assays. (A) NCI-N87 cells, which show higher expression of NPM, had a higher survival rate than SGC7901 cells, which exhibit lower NPM expression. (B) NPM overexpressing AGS cells had a significantly higher half maximal inhibitory value compared with the NPM-knockdown cells. NPM, nucleophosmin; Her-2, human epidermal growth factor receptor-2.
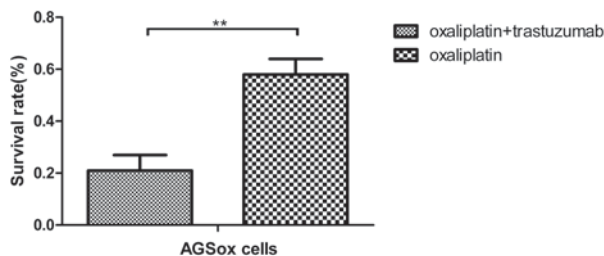

Figure 5. Trastuzumab sensitized gastric cancer cells to oxaliplatin *in vitro*. AGSox cells treated with oxaliplatin combined with trastuzumab exhibited a significantly lower survival rate than the cells treated with oxaliplatin alone. **$P<0.01$. AGSox cells, NPM-overexpressing AGS gastric cancer cells.

between Her-2 and NPM, transfection technology was used to alter their expression. First, SGC7901 and NCI-N87 cells were transfected with Her-2-siRNA or non-specific siRNA. Western blot analysis confirmed that Her-2 expression was successfully altered by the transfection, and that NPM expression changed in accordance with Her-2 downregulation (Fig. 3A). Conversely, no significant difference in the expression level of Her-2 was observed when NPM expression was silenced using NPM-siRNA (Fig. 3A). Furthermore, elevated NPM expression was observed following transfection of MKN-28 cells with pcDNA3.1-Her-2, whereas overexpression of NPM caused

by transfection of the MKN-28 cells with pcDNA3.1-NPM did not lead to a prominent change in Her-2 expression (Fig. 3B). These results suggest that NPM expression in gastric cancer cells is regulated by Her-2.

NPM overexpression correlates with resistance to oxaliplatin. Considering the opposite effects of NPM and oxaliplatin on DNA, we hypothesized that NPM may affect the sensitivity of gastric cancer cells to oxaliplatin. Three gastric cancer cell lines (SGC7901, AGS and NCI-N87), which show different expression levels of NPM, were treated with serial concentrations of oxaliplatin for 24 h, and the viability of these cells was assessed using CCK-8 assays. The results revealed that the NCI-N87 cells had the highest survival rate and highest expression levels of NPM, while the SGC7901 cells exhibited the lowest survival rate and the lowest NPM expression (Fig. 4A), indicating that the expression of NPM was positively correlated with oxaliplatin resistance. To further validate the results, AGS cells were transfected with pcDNA3.1-NPM or NPM-siRNA, and western blot analysis revealed that the transfection efficiently altered NPM expression. As shown in Fig. 4B, the cell viability was markedly lower in NPM-knockdown cells, whereas the cells overexpressing NPM exhibited a higher viability. Furthermore, the cells overexpressing NPM

had a significantly higher IC_{50} value, as compared with the NPM-knockdown cells (7.19 μg/ml vs. 2.26 μg/ml; Fig. 4B). These results suggest that NPM expression was associated with the therapeutic efficacy of oxaliplatin and that NPM may be a potential predictive factor of oxaliplatin resistance in gastric cancer.

Trastuzumab sensitizes gastric cancer cells to oxaliplatin in vitro. Considering the relationship between Her-2 and NPM, NPM-overexpressing cells (AGSox) were used to reveal the role of trastuzumab in the reversal of oxaliplatin resistance. The AGSox cells were incubated with oxaliplatin alone (2.4 μg/ml) or in combination with 5 μg/ml trastuzumab and then assessed for viability. The CCK-8 assay results demonstrated that the AGSox cells treated with oxaliplatin combined with trastuzumab exhibited a significantly lower survival rate than the cells that experienced monotherapy (21 vs. 58%, respectively; Fig. 5). These results suggest that there is a synergistic effect between the two drugs, and that the resistance of gastric cancer cells to oxaliplatin may be reversed through the inhibition of NPM via Her-2.

Discussion

The results of the present study provided evidence for a functional role of NPM in oxaliplatin resistance in human gastric cancer cells and clarified the relationship between NPM and Her-2 in gastric cancer, which has seldom been described. Notably, NPM and Her-2 were significantly upregulated in gastric cancer cell lines and clinical samples, as compared with two normal gastric epithelium cell lines and paracancerous tissues, respectively. Furthermore, a positive relationship between the expression levels of NPM and Her-2 was observed; when Her-2 expression was up- or down-regulated, the NPM expression was altered accordingly. Conversely, alterations in the expression levels of NPM did not significantly affect Her-2 expression, indicating that the expression of NPM was at least partially regulated by Her-2. In addition, NPM overexpression was shown to promote oxaliplatin resistance in gastric cancer cells and the Her-2-specific inhibitor trastuzumab was able to significantly reverse the oxaliplatin resistance.

Her-2 has an important role in promoting cell proliferation and suppressing apoptosis, and may facilitate uncontrolled cell growth and tumorigenesis by transmitting extracellular signals to initiate intracellular signaling pathways (25). Importantly, Her-2 overexpression has been described in a variety of tumors, including breast cancer, gastric cancer, non-small cell lung cancer and ovarian cancer (26). Overexpression of Her-2 at the protein and/or mRNA level is detected in a variable percentage of cases, depending on the tumor (27-29). Furthermore, unlike breast cancer, previous studies have reported inconsistent findings regarding the prognostic role of Her-2 in gastric cancer (30-32). A previous study demonstrated that patients with intestinal-type and well-differentiated gastric cancer have a higher rate of Her-2 positivity than patients with diffuse-type and poorly-differentiated cancer (29). It has been reported that the Her-2-positive status was associated with a significantly worse prognosis and to relevant clinicopathological characteristics, including already established prognostic factors such as serosal invasion and lymph node metastasis (33).

Ding *et al* (34) reported that NPM was an independent prognostic indicator for tumor recurrence in patients with gastric cancer, and was associated with advanced tumor stages and a poor prognosis. Our previous study demonstrated that NPM and Her-2 expression were closely related to a later TNM stage and the recurrence or metastasis of gastric cancer (22). However, the relationship between NPM and Her-2 has yet to be investigated. The results of the present study suggested that NPM expression was closely associated with the Her-2 expression status, and that NPM is a downstream cellular target of Her-2-dependent signaling. Furthermore, the results indicated that Her-2 and NPM may be important diagnostic markers for gastric cancer. However, the precise regulatory mechanisms underlying the interaction between Her-2 and NPM remain unclear.

Recurrence and subsequent acquired chemoresistance are responsible for therapeutic failure and poor improvement in the 5-year survival rate of gastric cancer patients (22). Oxaliplatin has been reported to induce apoptosis via various mechanisms, including downregulating the expression of anti-apoptotic proteins, influencing mitogen-activated protein kinase activities, inhibiting DNA-repair mechanisms and inducing mitotic catastrophe (35-38). However, chemoresistance has been reported for oxaliplatin (39), and is thought to occur as a result of Her-2 overexpression (40). Elucidating the mechanisms by which Her-2 mediates resistance to oxaliplatin may be crucial for improving therapeutic strategies. NPM is required for DNA-dependent activities, including transcription, replication and repair (41). On the basis of the opposite roles of NPM and oxaliplatin in the progression of DNA repair, the authors of the present study hypothesized that NPM may be involved in the resistance of gastric cancer to oxaliplatin and further examined this possibility. The present study found that NPM overexpression was closely related to oxaliplatin resistance in gastric cancer cells, whereas silencing of NPM with siRNA was able to significantly increase the chemosensitivity of gastric cancer cells, indicating that NPM may be a potential therapeutic target for oxaliplatin-resistant gastric cancer.

The present study also demonstrated that resistance to oxaliplatin induced by NPM overexpression was partially reversed by trastuzumab, which is an anti-Her-2 monoclonal antibody, indicating a synergistic antitumor effect for trastuzumab and oxaliplatin in Her-2-positive gastric cancer cells. Overexpression or amplification of Her-2 is detected in 7-34% of gastric cancer patients and, in a previous study, the addition of trastuzumab to chemotherapy improved survival in patients with advanced gastric cancer compared with chemotherapy alone (21). ToGA clinical trials in patients with gastric adenocarcinoma and gastroesophageal junction adenocarcinoma have shown a significant survival benefit for patients treated with a combination of trastuzumab and standard chemotherapy (42,43). The results of a multicenter phase II clinical trial suggested that the combination of trastuzumab and XELOX (oxaliplatin and capecitabine) was highly efficacious, demonstrating an objective response rate of 68%, a median progression-free survival (PFS) of 9.8 months and a median overall survival of 21.0 months; the PFS was enhanced by 2 months compared with previous phase II trials of XELOX alone (44,45). Furthermore, trastuzumab showed synergistic cytotoxic effects with oxaliplatin on

Her-2-positive gastric and breast cancer cells (46). However, adverse effects, including grade 3-4 peripheral neuropathy, neutropenia and anemia, were noted following combined treatment with trastuzumab and oxaliplatin (47). Therefore, further investigations are required to identify a more efficient and safe therapy.

In conclusion, the present study demonstrated that NPM may be a potential target for oxaliplatin-resistant gastric cancer. In addition, trastuzumab and oxaliplatin exhibited a synergistic antitumor effect in Her-2-postive gastric cancer cells.

Acknowledgements

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