NIK- and IKKβ-binding protein contributes to gastric cancer chemoresistance by promoting epithelial-mesenchymal transition through the NF-κB signaling pathway

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Abstract. Systematic chemotherapy is indispensable for gastric cancer patients with advanced stage disease, but the occurrence of chemoresistance drastically limits treatment effectiveness. There is a tremendous need for identifying the underlying mechanism of chemoresistance. NIK- and IKKβ-binding protein (NIBP) (also known as TRAPPC9, trafficking protein particle complex 9) is a regulator of the cytokine-induced NF-κB signaling pathway which has been proven to play pivotal roles in the progression of various malignancies. Nevertheless, it is still ambiguous whether NIBP is involved in the chemoresistance of gastric cancer. The aim of the present study was to investigate the effect of NIBP on chemotherapy resistance of gastric cancer (GC) and to research the mechanisms of Ginkgo biloba extract 761 (EGb 761®) on reversing chemoresistence which has been confirmed in our previous study. In the present study, the results of immumohistochemisty revealed that the positive staining rates of NIBP, NF-κB p65 and NF-κB p-p65 in gastric cancer tissues were obviously higher than those in normal tissues. Furthermore, a close correlation was found to exist between the expression of NIBP and NF-κB p65 (p-p65) in gastric cancer tissues. Moreover, the overexpression of NIBP was closely related to tumor differentiation, depth of invasion, clinical stage and lymphatic metastasis in gastric cancer. Western blot analysis, real-time PCR, MTT assay and flow cytometric analysis were performed and the results demonstrated that compared with the gastric cancer SGC-7901 cells, the expression of NIBP, NF-κB p65, NF-κB p-p65 and mesenchymal marker vimentin were significantly increased in gastric cancer multidrug-resistant SGC-7901/CDDP cells, and the epithelial cell marker ZO-1 was significantly decreased. Meanwhile, it was found that SGC-7901/CDDP cells were accompanied by spindle-like mesenchymal appearance and upregulation of stem cell marker CD133 which has been verified to be an upstream regulatory gene of epithelial-mesenchymal transition (EMT). Further research confirmed that downregulation of NIBP by Ginkgo biloba extract (EGb) 761 EGb 761 suppressed the *cis*-diamm inedichloroplatinum(II) (CDDP)-induced NF-κB signaling pathway, EMT and the expression of CD133 in SGC-7901 and SGC-7901/CDDP cells. Altogether, these data indicate that the NIBP-regulated NF-κB signaling pathway plays a pivotal role in the chemoresistance of gastric cancer by promoting CD133-induced EMT.

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Introduction

Gastric cancer (GC) is one of the most common digestive system tumors and patients suffering from GC are usually diagnosed with late-stage disease. Systemic chemotherapy is the most effective treatment to prolong patient survival and improve the quality of life of patients with advanced GC. Yet, the occurrence of resistance to chemotherapy drugs greatly reduces the clinical efficacy of chemotherapy in GC (1). Thus, identification of potential biomarkers of adverse survival outcomes as well as an in-depth study of the mechanisms underlying drug resistance are crucial for the treatment of GC.

NIK- and IKKβ-binding protein (NIBP), also known as trafficking protein particle complex 9 (TRAPPC9), is a type of NF-κB signaling pathway regulating factor that has been detected in human nerve cells (2-4). Moreover, the NF-κB signaling pathway is a pivotal mediator for both physiological and pathological events in cell proliferation, apoptosis as well as in oncogenesis (5,6). Hence, NIBP may regulate activation of the NF-κB signaling pathway in tumorigenesis as well as in progression. Previous studies have demonstrated that NIBP is aberrantly and highly expressed in colorectal cancer, while

overexpression of NIBP was found to be significantly correlated with tumor differentiation, clinical stage and metastasis (7,8). Additionally, it was found that lentiviral-mediated silencing of NIBP expression inhibited colon cancer cell growth, invasion and metastasis, and this may be related to regulation of the activation of the NF-κB signaling pathway (9). Nevertheless, little is known concerning the functional relevance of NIBP expression, in particular, the effect of NIBP on the chemoresistance in GC.

Epithelial-mesenchymal transition (EMT) is the process whereby epithelial cells undergo the dissolution of cell-cell adhesion and loss of apico-basolateral polarity and transform into mesenchymal cells. Downregulation of epithelial markers and upregulation of mesenchymal markers are the most conspicuous molecular characteristics of EMT. It has been confirmed that EMT is responsible for the progression of various types of malignancies in pathological conditions (10). Emerging evidence indicates that EMT not only promotes migratory and invasive capacity of cancer cells, but it is also the key to acquisition of chemoresistance (11). Furthermore, suppression of the process of EMT was found to improve sensitivity to chemotherapeutics in genetically engineered mouse models with deletion of Snail or Twist (12). Research has shown that EMT is induced by activation of the NF-κB signaling pathway, resulting in cisplatin-treatment resistance of bladder cancer (13). Our previous research indicated that the chemotherapy sensitivity of GC cells was enhanced through suppression of the NF-κB signaling pathway following treatment with Ginkgo biloba extract 761 (EGb 761) (14).

Cancer stem cells (CSCs) are a subgroup of cells which possess self-renewal capacity, drive tumorigenesis, maintenance, relapse and therapy resistance (15). CD133 is a surface glycoprotein which has been recognized as a universal CSC marker in many types carcinomas. Accumulating evidence indicates that CD133 is also a functional factor involved in tumorigenesis and tumor progression (16). It has been reported that CD133 overexpression facilitated tumor invasiveness and metastasis by induction of EMT, which was found to be related to activation of the NF-κB signaling pathway in pancreatic cancer (17). In addition, following the deletion of CD133 by ultrasound-targeted microbubble destruction technique (UTMD), EMT was significantly inhibited with the accompanying suppression of the NF-κB signaling pathway *in vitro* and *in vivo* (18). The above studies demonstrate that CD133 serves as a critical activation factor to participate in constitution of the EMT regulatory network of the NF-κB signaling pathway. In the light of these results, it was hypothesized that NIBP may activate the NF-κB signaling pathway and contribute to the chemoresistance of GC by regulating CD133-induced EMT. Meanwhile, EGb 761 may reverse drug resistance through the suppression of EMT regulated by NIBP-mediated NF-κB signaling pathway.

Materials and methods

Drug preparation and cell culture. EGb 761® was purchased from Dr Willmar Schwabe Pharmaceuticals (Karlsruhe, Germany). Human gastric cancer cell line SGC-7901 and CDDP-resistant gastric cancer cell line SGC-7901/CDDP were provided by the Chinese Academy of Sciences (Beijing, China). The cells were grown in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal calf serum (FCS; Gibco; Thermo Fisher Scientific), 100 U/ml penicillin and 100 μg/ml streptomycin, at 37˚C in a 5% CO_2 humidified atmosphere. SGC-7901/CDDP cells were conventionally maintained in the above RPMI-1640 medium containing 5 μg/ml *cis*-diammin-edichloroplatinum (II) (CDDP) (Hospira Australia Pty Ltd., Mulgrave, VIC, Australia).

Clinical data. Forty patients with GC who underwent curative resection were enrolled in the present study from January 2016 to January 2017 at The First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi, China). There were 30 males and 10 females among the 40 patients and the age of the patients ranged from 35 to 74 years. Written informed consent was provided by each patient. Samples of tumor and normal gastric tissues were obtained at the time of surgery; for normal tissue collection, samples were taken at least 10 cm from the gastric cancer edge. The specimens were fixed using 4% paraformaldehyde. After dehydration, transparent and paraffin-embedding, the specimens were constructed into 4-μm-thick serial section. For all specimens, a definite diagnosis of GC was made by pathology. The research was authorized by the Medical Ethics Committee of The First Affiliated Hospital of Guangxi Medical University, Guangxi, China.

Immumohistochemical staining. Tissue sections were fixed with 0.01 mol/l citric acid for 5 min and blocked with normal goat serum. The sections were then incubated with rabbit polyclonal anti-NIBP (1:200; cat. no. 16014-1-AP; Proteintech Group, Wuhan, China), rabbit monoclonal anti-NF-κB p65 (1:200; cat. no. 8242; Cell Signaling Technology, Beverly, MA, USA) and rabbit polyclonal anti-NF-κB p-p65 antibodies (1:200; cat. no. 3031; Cell Signaling Technology) for 1 h at room temperature, and more steps were strictly complied with according to the SP immunohistochemical kit (ZSGB Biotech, Beijing, China) instructions. Positive control was provided by the manufacturers, and using phosphate-buffered saline (PBS) instead of primary antibody as a negative control. The positive staining was evaluated by combining staining intensity with percentage of positive cells to conduct an analysis of half quantitative. The intensity of staining was scored as: no staining, 0; light yellow, 1; yellow, 2; and brown, 3. The percentage of positive cells was scored as: positive cells <5%, 0; positive-staining cells 5-25%, 1; positive cells 26-50%, 2; and positive cells >50%, 3. A weighted score was produced by adding the scores of the staining intensity and the percentage of positive cells. The weighted scores ≤2 were defined as negative; >2 were defined as positive.

Cell viability analysis. Effects of EGb 761 and CDDP on cell viability were assessed by using MTT assay. MTT was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Briefly, cells were cultured in 96-well plate and exposed to various concentrations of EGb 761 and/or CDDP for 24 h, the culture medium was discarded and 20 ml of MTT (5 mg/ml) was added to each well for 4 h. The formazan crystals were then dissolved in dimethyl sulfoxide

Table I. Expression of NIBP, NF-κB p65 and p-p65 in the gastric cancer and normal gastric tissues.

		NIBP+		NF-κB p65+		NF-κB p-p65+	
Samples	N	n (%)	P-value	n (%)	P-value	n (%)	P-value
Normal tissues	40	14 (35)	0.002	17 (42.5)	0.006	15 (37.5)	0.007
Cancer tissues	40	29 (72.5)		30 (75)		28 (70)	

NIBP, NIK- and IKKβ-binding protein.

(DMSO; Sigma-Aldrich; Merck KGaA) and the absorbance was assessed at 570 nm with a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Real-time fluorescent quantitative PCR. SGC-7901 cells were treated with 2 μg/ml CDDP with or without EGb 761 (360 μg/ml) for 24 h, and SGC-7901/CDDP cells were treated in 4 μg/ml CDDP with or without EGb 761 (360 μg/ml) for 24 h. RNA was extracted from cells by using TRIzol reagent (Takara Biotechnology, Co., Ltd., Dalian, China). Then, 1 μg DNAse I-treated RNA was used to synthesis cDNA for the subsequent real-time fluorescence PCR amplification according to the Reverse Transcription kit (Takara Biotechnology, Co., Ltd.) instructions. The specific primer sequences for NIBP were 5'-GAACTGCCTTAGCCCTGAAGACAT A-3' (forward) and 5'-AGCCTTGATGCACGCTTCC-3' (reverse), generating a fragment of 109 bp. The specific primer sequences for NF-κB p65 were 5'-ACCTCGACGCATTGCTGTG-3' (forward) and 5'-CTGGCTGATCTGCCCAGAAG-3' (reverse), generating a fragment of 145 bp. The primers for β-actin were 5'-GCACCG TCAAGGCTGAGAAC-3' (forward) and 5'-TGGTGAAGA CGCCAGTGGA-3' (reverse), generating a fragment of 138 bp. The SYBR Green PCR Master Mix kit (Takara Biotechnology, Co., Ltd.) was used and quantitative PCR analysis was carried out on the LightCycler 480 (Roche Diagnostics, Indianapolis, IN, USA). The extent of NIBP and NF-κB p65 mRNA expression were quantitated by using Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA).

Western blot analysis. SGC-7901/CDDP and SGC-7901 cells were pretreated with the appropriate concentration of EGb 761 or CDDP (refer to real-time fluorescent quantitative PCR section). Related proteins from the SGC-7901 and SGC-7901/CDDP cells were prepared using a Beyotime Biotechnology cytoplasmic kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the kit instructions. All proteins were separated on SDS-PAGE gels for electrophoresis, and transferred onto polyvinylidene fluoride (PVDF) membranes. Then, the membranes were blocked with PBST (PBS containing 0.05% Tween-20) containing 5% non-fat dry milk for 1 h, and each membrane was incubated with rabbit polyclonal anti-NIBP (1:1,000; cat. no. 16014-1-AP; Proteintech Group), rabbit monoclonal anti-NF-κB p65 (1:1,000; cat. no. 8242; Cell Signaling Technology), rabbit polyclonal anti-NF-κB p-p65 (1:1,000; cat. no. 3031; Cell Signaling Technology), rabbit monoclonal anti-vimentin antibody (1:1,000; cat. no. 5741; Cell Signaling Technology), rabbit polyclonal anti-ZO-1 (1:500; cat. no. 21773-1-AP; Proteintech Group), rabbit polyclonal anti-CD133 (1:500; cat. no. 18470-1-AP; Proteintech Group) and rabbit polyclonal anti-GAPDH antibody (1:5,000; cat. no. 10494-1-AP; Proteintech Group) overnight at 4˚C. Finally, the membranes were probed with the appropriate secondary antibodies (1:500; cat. no. E032720; EarthOx, San Francisco, CA, USA) for 1 h at room temperature. The generating protein bands were visualized and analyzed by Odyssey CLx Infrared Imaging system (LI-COR Biosciences, Lincoln, NE, USA).

Flow cytometric analysis. Cells were seeded in 6-well plates at $5x10^5$ cells/well, starved overnight in RPMI-1640 medium with 0.5% serum and incubated with the appropriate concentration of EGb761 or CDDP (refer to real-time fluorescent quantitative PCR section). Then, the cells were collected and washed with cold PBS twice. The cells were then gently resuspended in 400 μl binding buffer, and 5 μl Annexin V-FITC was added to the above cell solution. The cells were gently vortexed, incubated for 10 min at 4˚C avoiding light; 10 μl propidium iodide (PI) was added and the cells were cultured for another 5 min. Samples were analyzed using FACSCalibur flow cytometry (BD Biosciences, San Jose, CA, USA) and CellQuest software (BD Biosciences) was used to analyze the results.

Statistical analysis. All results are presented as the mean ± SD. All the experiments were repeated at least three times. SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. The data were analyzed using the Student's t-test or ANOVA test. The frequencies in the different groups were evaluated using the Fisher's exact test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Immunohistochemistry analysis of NIBP, NF-κB p65 and p-p65 in GC tissues. Immunohistochemical analysis of tissue sections showed that positive NIBP staining was mainly observed in the cytoplasm, and positive NF-κB p65 and p-p65 staining was detected in the cell nucleus and cytoplasm (Fig. 1). The percentages of positive expression of NIBP, NF-κB p65 and p-p65 in the GC tissues were significantly higher than those determined in the normal tissues (Table I).

Clinical significance of NIBP, NF-κB p65 and p-p65 in GC tissues. As shown in Table II, the expression levels of

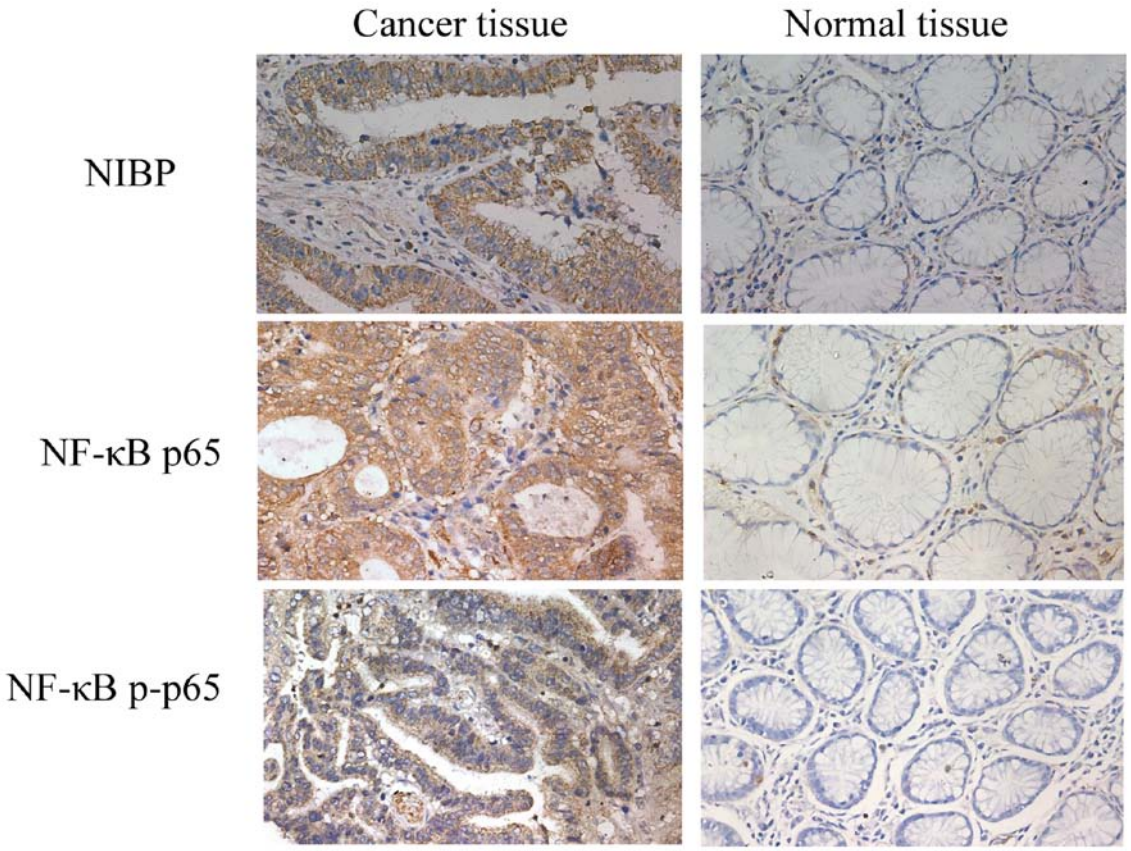

Figure 1. The immunohistochemical staining analysis of NIBP, NF-κB p65 and p-p65 in gastric cancer and normal tissues (magnification, x400).

Table II. Correlation of the clinicopathological characteristics of the gastric cancer tissues and NIBP, NF-κB p65 and p-p65 expression.

		NIBP			NF-κB p65			NF-κB p-p65		
Clinicopathological characteristics	N	-	+	P-value	-	+	P-value	-	+	P-value
Age (years)				0.455			0.103			>0.999
<50	11	4	7		5	6		3	8	
≥50	29	7	22		5	24		9	20	
Sex				0.418			0.232			>0.999
Male	30	7	23		6	24		9	21	
Female	10	4	6		4	6		3	7	
Serosal invasion				0.029			0.018			0.011
Negative	14	7	7		7	7		8	6	
Positive	26	4	22		3	23		4	22	
Histological grade				0.006			0.025			0.018
Well and moderately differentiated	18	9	9		8	10		9	9	
Poorly differentiated	22	2	20		2	20		3	19	
TNM stage				0.012			0.028			0.005
I+II	19	9	10		8	11		10	9	
III+IV	21	2	19		2	19		2	19	
Lymph node metastasis				0.014			0.007			0.041
Negative	10	6	4		6	4		6	4	
Positive	30	5	25		4	26		6	24	

NIBP, NIK- and IKKβ-binding protein.

Table III. Correlation between the expression of NF-κB p65 and p-p65 and NIBP in the gastric cancer tissues.

	NF-κB p65				NF-κB p-p65			
	-	+	Correlation	P-value	-	+	Correlation	P-value
NIBP				<0.001				<0.001
-	7	4	0.550		8	3	0.574	
+	3	26			4	25		

NIBP, NIK- and IKKβ-binding protein

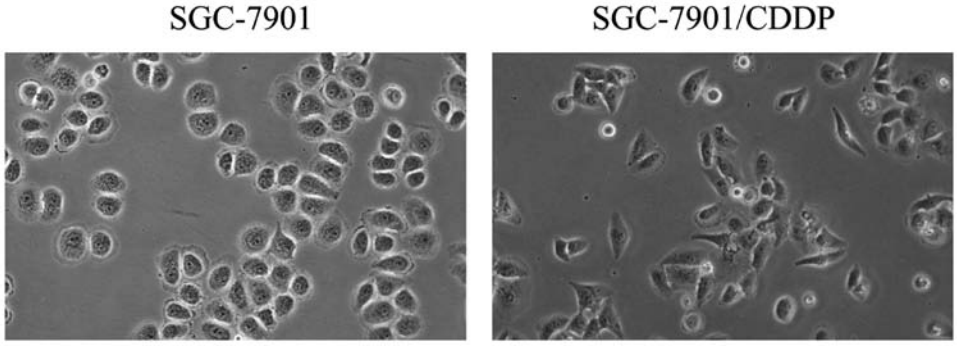

Figure 2. Morphological differences between SGC-7901 and SGC-7901/CDDP cells were observed by phase contrast microscopy (magnification, x400).

NIBP, NF-κB p65 and p-p65 were closely related to tumor invasion depth, differentiation degree, pathological stage and lymphatic metastasis. Furthermore, there was a closely correlation between the expression of NIBP and NF-κB p65 (p-p65) (Table III) in GC tissues.

Expression levels of NIBP, NF-κB p65, p-p65, vimentin, ZO-1 and CD133 in GC cells. Morphological observation displayed huge morphological differences between SGC-7901/CDDP cells which exhibited spindle-like mesenchymal appearance and SGC-7901 cells which displayed cobblestone-like epithelial shape (Fig. 2).

The results indicated that the mRNA expression levels of NIBP and NF-κB p65 in the SGC-7901/CDDP cells were enhanced as compared to those of SGC-7901 cells (Fig. 3). In line with the results of real-time PCR, the protein expression of NIBP, NF-κB p65 and p-p65 in SGC-7901/CDDP cells was higher than levels noted in the SGC-7901 cells (Fig. 4A) (P<0.01). Compared with the SGC-7901 cells, the expression levels of vimentin and CD133 were increased in the SGC-7901/CDDP cells, while the expression of ZO-1 was decreased (Fig. 4B).

Effects of EGb 761 on the expression of NIBP, NF-κB p65, p-p65, CD133 and EMT induced by CDDP. Compared with the control group, the mRNA expression levels of NIBP and NF-κB p65 were significantly increased in the CDDP group, and following combination treatment of EGb 761 and CDDP, the mRNA expression of NIBP and NF-κB p65 induced by CDDP were significantly attenuated in both cell lines (Fig. 5). Consistently, the protein expression levels of NIBP, NF-κB p65 and p-p65 were increased after treatment with CDDP and decreased following the combined treatment with EGb 761 and CDDP (Fig. 6).

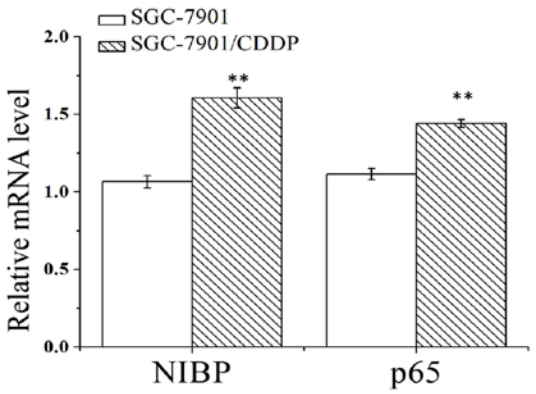

Figure 3. The mRNA expression of NIBP and NF-κB p65 in SGC-7901 and SGC-7901/CDDP cells. **P<0.01 vs. the SGC-7901 group.

The protein expression of vimentin and CD133 were increased, while the expression of ZO-1 was decreased in the CDDP group, as compared to that in the control group. Following the combination treatment with EGb761 and CDDP, the expression levels of vimentin and CD133 induced by CDDP were significantly decreased, while expression of ZO-1 suppressed by CDDP was significantly increased (Fig. 7).

Effect of CDDP and EGb761 on the proliferation and apoptosis of GC cells. The proliferation of SGC-7901 and SGC-7901/CDDP cells were markedly suppressed following treatment with

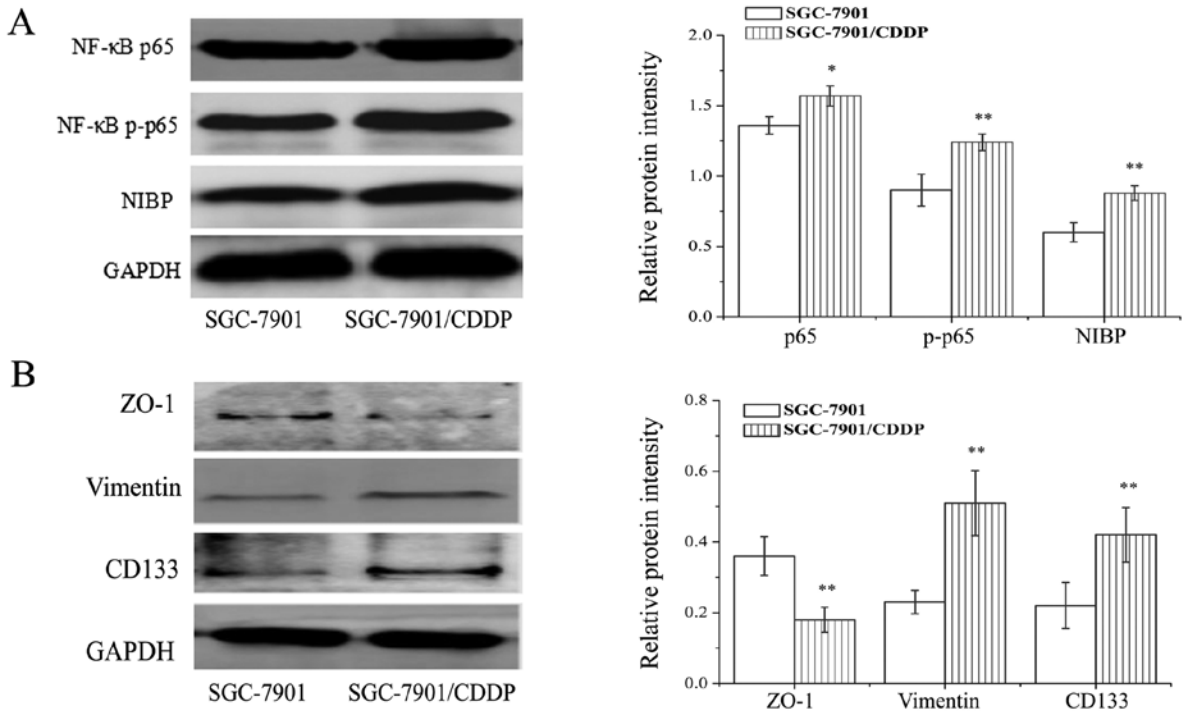

Figure 4. The protein expression levels of NIBP, NF-κB p65, NF-κB p-p65, CD133 and EMT markers in gastric cancer cells. (A) NIBP, NF-κB p65 and p-p65 protein expression in SGC-7901 and SGC-7901/CDDP cells. (B) ZO-1, vimentin and CD133 protein expression in SGC-7901 and SGC-7901/CDDP cells. *P<0.05 or **P<0.01 vs. the SGC-7901 group.

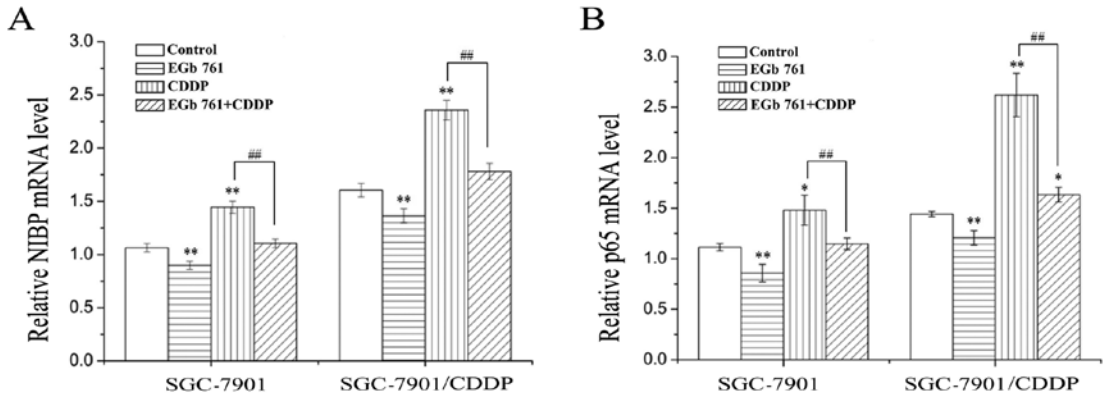

Figure 5. Effect of EGb761 and CDDP on the mRNA expression of NIBP and NF-κB p65 in gastric cancer cells. Cells were treated with appropriate concentration of EGb761 and/or CDDP for 24 h (refer to Real-time fluorescent quantitative PCR section). (A) The real-time PCR analysis of NIBP expression in SGC-7901 and SGC-7901/CDDP cells. (B) The real-time PCR analysis of NF-κB p65 expression in SGC-7901 and SGC-7901/CDDP cells. *P<0.05 or **P<0.01 vs. the control group. $^{\#\#}$P<0.01 vs. the CDDP group.

CDDP or EGb761 in a dose-dependent manner, and the proliferation inhibition of CDDP in SGC-7901 cells was significant greater than that of SGC-7901/CDDP cells (Fig. 8A). However, there was no significant difference between the proliferation inhibition of EGb761 in the SGC-7901 and SGC-7901/CDDP cells (Fig. 8B), suggesting that SGC-7901/CDDP cells were not resistant to EGb761. The proliferation inhibition of CDDP was significantly enhanced by the combined treatment with EGb761 in a dose-dependent manner in both cell lines (Fig. 8C and D). The flow cytometric analysis revealed that the cell apoptosis induced by CDDP was elevated following the combined treatment with EGb761 and CDDP in both cell lines (Fig. 9).

Discussion

NIK- and IKKβ-binding protein (NIBP) (also known as TRAPPC9, trafficking protein particle complex 9), as a significant transcription protein interacting with NIK and IKKβ, plays a crucial role in a number of fundamental pathophysiological processes. Increasing evidence indicates that NIBP overexpression is seemingly involved in the genesis and development of breast cancer and colon cancer by activation of the NF-κB signaling pathway (7,9). In the present study, NIBP was found extensively expressed in gastric cancer (GC) tissues and there was a closely correlation between the overexpression of NIBP and tumor invasion depth, differentiation

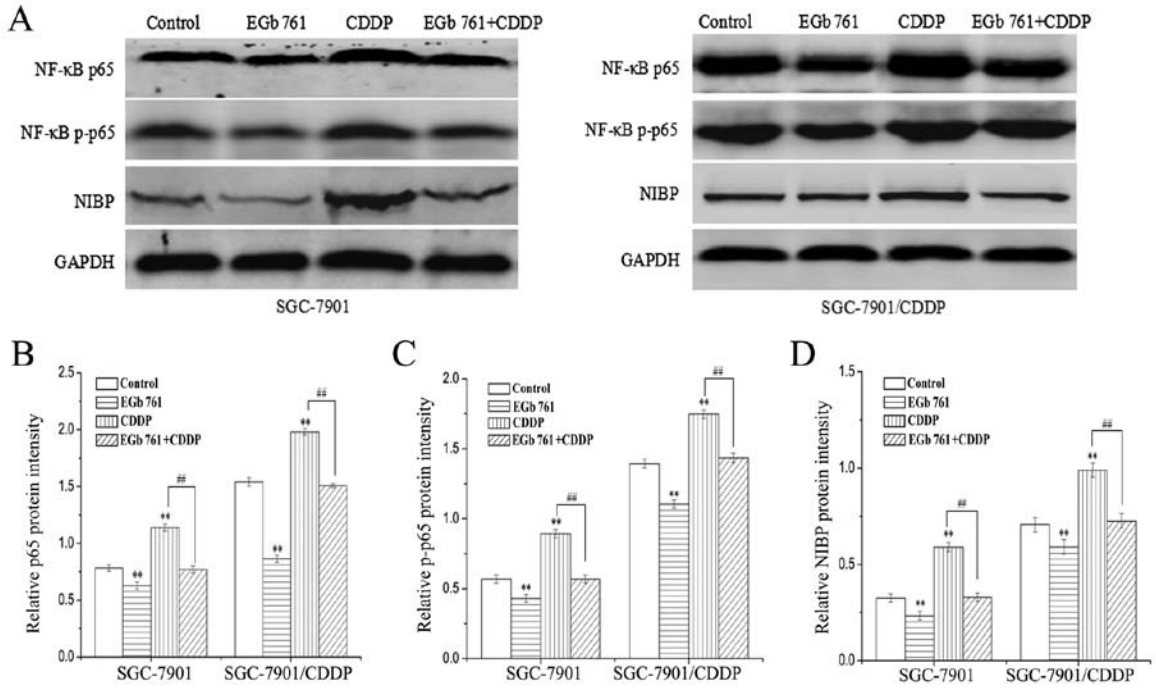

Figure 6. Effect of EGb761 and CDDP on the protein expression of NIBP, NF-κB p65 and p-p65 in gastric cancer cells. Cells were treated with the appropriate concentration of EGb761 and/or CDDP for 24 h (refer to Real-time fluorescent quantitative PCR section). (A) Western blot analysis of NIBP, NF-κB p65 and p-p65 in SGC-7901 and SGC-7901/CDDP cells. (B-D) Quantity of the relative expression level of NIBP, NF-κB p65 and NF-κB p-p65. **P<0.01 vs. the control group. $^{\#\#}$P<0.01 vs. the CDDP group.

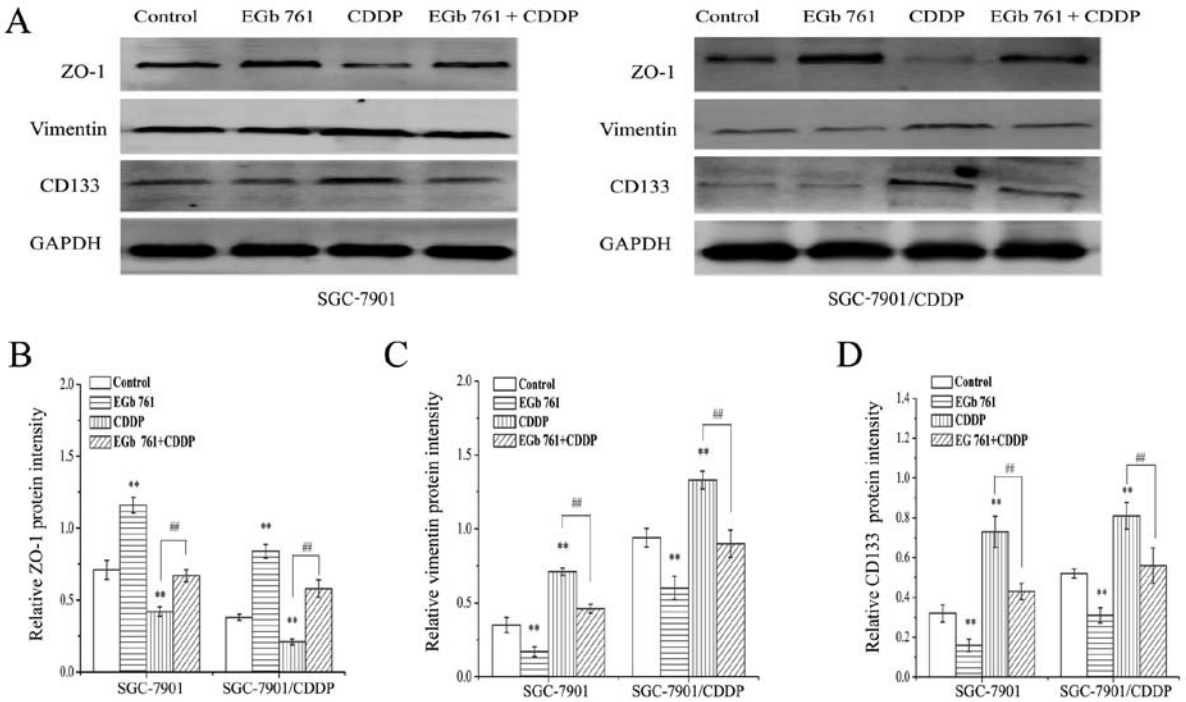

Figure 7. Effect of EGb761 and CDDP on the protein expression of ZO-1, vimentin and CD133 in gastric cancer cells. Cells were treated with the appropriate concentration of EGb761 and/or CDDP for 24 h (refer to Real-time fluorescent quantitative PCR section). (A) Western blot analysis of ZO-1, vimentin and CD133 in SGC-7901 and SGC-7901/CDDP cells. (B-D) Quantity of the relative expression level of ZO-1, vimentin and CD133. **P<0.01 vs. the control group. $^{\#\#}$P<0.01 vs. the CDDP group.

degree, clinical stage and lymphatic metastasis. Thus, NIBP may contribute to the oncogenesis and progression of gastric carcinoma. Our previous studies confirmed that NIBP expression is linked with tumor differentiation, clinical stage and metastasis in colorectal cancer (7,8). Further research confirmed that the expression of NF-κB p65 and NF-κB p-p65 is closely related to the overexpression of NIBP in GC tissues. Hence, NIBP overexpression may exert negative influence

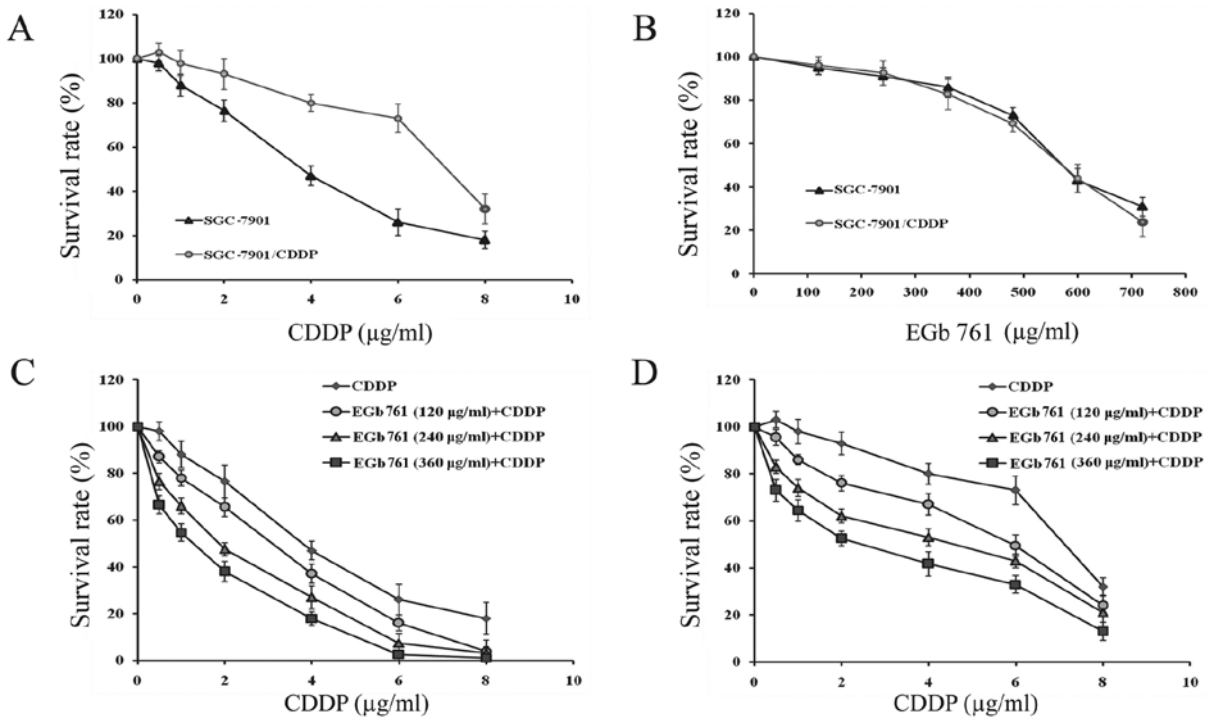

Figure 8. EGb761 enhances the proliferation inhibition effect of CDDP in gastric cancer cells. (A) Effect of CDDP on cell proliferation in the SGC-7901 and SGC-7901/CDDP cells. Cells were only treated with different concentrations of CDDP for 24 h. (B) Effect of EGb761 on cell proliferation in the SGC-7901 and SGC-7901/CDDP cells. Cells were only treated with different concentrations of EGb761 for 24 h. (C and D) Effect of EGb761 combined with CDDP on the cell proliferation in the SGC-7901 and SGC-7901/CDDP cells. Cells were treated with 120, 240 and 360 μg/ml EGb761 and CDDP for 24 h.

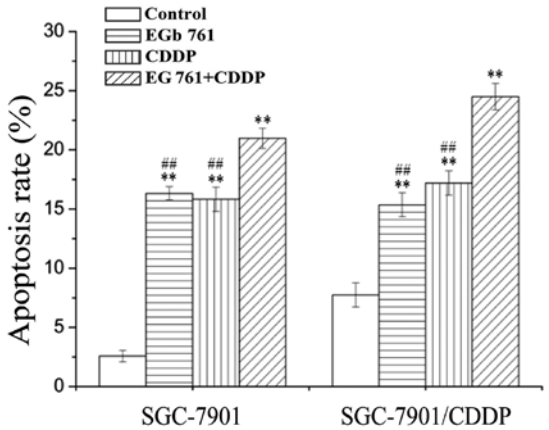

Figure 9. EGb761 elevates the apoptosis-inducing ability of CDDP in gastric cancer cells. SGC-7901 and SGC-7901/CDDP cells were treated with the appropriate concentration of EGb761, CDDP or EGb761+CDDP for 24 h (refer to real-time fluorescent quantitative PCR section) and the apoptotic rate was determined. **P<0.01 vs. control group. $^{\#\#}$P<0.01 vs. CDDP group.

on the prognosis of GC by regulating the NF-κB signaling pathway.

In cancer cells, the NF-κB signaling pathway is involved in cell proliferation, metastasis and chemotherapy resistance (19,20). Previous evidence indicates that an increase in the expression of IKKβ and NF-κB p-p65 was promoted with the expression of NIBP, which indicates that NIBP serves as an activator in the NF-κB canonical pathways (2). In the present study, the NF-κB signaling pathway was markedly activated by NIBP in the drug-resistant GC cells, which indicated that NIBP is involved in chemotherapy resistance in GC cells. Similar findings were reported in mouse intestinal nerve cells. The activation of NF-κB p65 was enhanced with the overexpression of NIBP, and the activation of NF-κB p65 was inhibited with the suppression of NIBP expression by using shRNA (4).

Epithelial-mesenchymal transition (EMT) is a process whereby epithelial cells depolarize and lose intercellular adhesion components and tight junction, with a corresponding phenotypic conversion from an epithelial phenotype into a fibroblast-like mesenchymal morphology (10). In the present study, SGC-7901 cells exhibited a cuboidal or cobblestone shape, however, SGC-7901/CDDP cells displayed a distinct elongated spindle or shuttle appearance. Furthermore, prominent cisplatin resistance was exhibited in the SGC-7901/CDDP cells, along with decreased expression of epithelial cell adhesion molecule ZO-1 and increased expression of mesenchymal cell marker vimentin. Research has indicated that EMT confers chemotherapy refractory properties to various types of malignancies and increases resistance against chemotherapeutics (11,21). Blocking EMT by knockdown of Snail effectively sensitizes cancer cells to chemotherapy and radiotherapy (22). Our previous study showed that EGb 761 enhanced the chemotherapy sensitivity of GC cells via suppression of the NF-κB signaling pathway (14). In the present study, it was confirmed that the chemo-refractory phenotype was reversed by EGb 761 by suppressing NIBP, the NF-κB signaling pathway and EMT. Research suggests that the activation of NF-κB weakens the effects of chemotherapy drugs on tumor cells and that it is associated with enhanced multi-drug resistance (23,24). Furthermore, cisplatin results in acquisition of chemoresistance via triggering EMT through the NF-κB signaling

pathway (12,25). Therefore, the NIBP/NF-κB/EMT axis may be involved in the chemoresistance of GC.

CD133, a robust CSC marker, impacts patient prognosis and chemosensitivity of gastric carcinoma and pancreatic cancer (16,26). It was found that the expression of CD133 was markedly increased in SGC-7901/CDDP cells when compared with that in the SGC-7901 cells in the present study. Compelling evidence indicates that CD133 acts as a upstream promoter of EMT progression, rather than just a stem cell marker (27). CD133 modulates EMT to exert its molecular function in pancreatic cancer and non-small cell lung cancer (28,29). These findings demonstrated that CD133-modulated EMT may be critical for developing chemoresistance in GC. In the present study, CDDP-induced expression of CD133 and EMT was suppressed following inhibition of NIBP-mediated NF-κB signaling pathway by combination treatment of EGb 761 and CDDP. Growing evidence suggests that CD133 expression induces EMT through activation of the NF-κB signaling pathway in tumors (17,18). Taken together, overexpression of NIBP may endow GC cells the capability of chemo-refractory properties via the NF-κB/CD133/EMT cascade.

Summarily, the NIBP-mediated NF-κB signaling pathway may contribute to oncogenesis, progression and chemotherapeutic drug resistance by mediating EMT in GC. Inhibition of NIBP may reverse drug resistance via the NF-κB/CD133/EMT cascade in gastric cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SQL and JAH conceived and designed the study. ZHF, LYZ, WHW and NQ performed the experiments. ZHF and SQL wrote the paper. ZHF, SQL, CYX, MBQ and LYZ reviewed and edited the manuscript. All authors read and approved the manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The research was authorized by the Medical Ethics Committee of The First Affiliated Hospital of Guangxi Medical University, Guangxi, China. Written informed consent was provided by each patient.

Consent for publication

This manuscript is approved by all participants for publication.

Competing interests

The authors declare that they have no competing interests.

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