ANTICANCER RESEARCH *40*: 1367-1374 (2020)
doi:10.21873/anticanres.14078

# RNF126 as a Marker of Prognosis and Proliferation of Gastric Cancer

KAZUHIRO MIGITA, SOHEI MATSUMOTO, KOHEI WAKATSUKI, TOMOHIRO KUNISHIGE, HIROSHI NAKADE, SHINTARO MIYAO and MASAYUKI SHO

*Department of Surgery, Nara Medical University, Nara, Japan*

**Abstract.** *Background/Aim: Ring finger protein 126 (RNF126) belongs to the family of RING E3 ubiquitin ligases. Although RNF126 has been reported to be overexpressed in several cancers, the role of RNF126 in gastric cancer remains unclear. Materials and Methods: We investigated the RNF126 expression in 170 primary gastric cancer tissues by immunohistochemistry, and explored its prognostic impact. The effect of the RNF126 expression on the proliferation of cancer cells was evaluated in vitro. Results: The RNF126 expression was significantly associated with tumor depth and presence of venous invasion. The RNF126 status was identified as an independent prognostic factor ($p<0.001$). RNF126 gene silencing significantly inhibited the proliferation of gastric cancer cells, induced $G_1$ phase arrest and increased the p21 protein level. Conclusion: RNF126 expression has a significant prognostic value in gastric cancer. RNF126 may play an important role in tumor progression of gastric cancer.*

Gastric cancer (GC) is one of the most common causes of cancer-related death in the world (1, 2). Despite several advancements in the diagnosis, surgical techniques and chemotherapy, a number of patients, particularly those with advanced disease, present with recurrence following curative resection (3-5). Thus, novel therapeutic strategies for GC are required. In addition, identifying potential biomarkers to predict the outcomes of patients is important to improve the prognosis of GC patients.

The ubiquitin-proteasome system plays an important role in controlling protein turnover and regulating various signaling pathways and cellular processes (6), and is cascaded by three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) (7, 8). Dysfunction of the ubiquitin system has been suggested to cause or contribute to the development and progression of cancer (9). To date, the upregulation or down regulation of E3 ubiquitin ligases (*e.g.*, Skp2, RBX1 and FBXW7) has been reported in GC (10-13).

Ring finger protein 126 (RNF126) belongs to the family of RING (really interesting new gene) E3 ubiquitin ligases, and has been identified as a ligase that promotes cancer cell proliferation through functional small interfering RNA (siRNA) library screening (14). RNF126 has been suggested to be involved in diverse biological phenomena, including the cell cycle, DNA repair, sorting of the cell surface membrane and protein quality homeostasis control (14-17). The overexpression of RNF126 has been identified in various cancer cell lines (*e.g.*, breast cancer, tongue cancer, lung cancer and prostate cancer) and human primary cancer tissues (*e.g.*, breast cancer and ovarian cancer) (14, 18-21). However, the functions and role of RNF126 in cancer still remain unclear. Moreover, there are only limited data on the expression and clinical relevance of RNF126 in cancer. In the present study, we investigated the expression, clinical significance and prognostic impact of RNF126 in GC. Furthermore, we evaluated the effect of the RNF126 expression on the proliferation of GC cells using an RNA interference method *in vitro*.

## Materials and Methods

*Clinical samples.* One hundred seventy GC samples were obtained from the resected specimens, and then fixed in 10% phosphate-buffered formalin and embedded in paraffin. All patients underwent curative gastrectomy in the Department of Surgery, Nara Medical University, between January 2004 and December 2008. These patients had not undergone preoperative chemotherapy or radiotherapy. The tumor stage was determined according to the TNM classification system (22). Follow-up was continued until death or January 2016. The median follow-up period was 61.2 months. Written informed consent was obtained from all patients before the operation, according to our institutional guidelines. This study was approved by the Local Ethics Committee on Clinical Investigation of Nara Medical University Hospital (no. 1193-2).

*Correspondence to:* Kazuhiro Migita, Department of Surgery, Nara Medical University, 840 Shijo-cho, Kashihara, Nara, 634-8522, Japan. Tel: +81 744298863, Fax: +81 744246866, e-mail: kmigita@naramed-u.ac.jp

*Key Words:* RNF126, E3 ubiquitin ligase, gastric cancer.

*Immunohistochemistry*. The sections were stained using a DAKO EnVision system (Dako Cytomation, Kyoto, Japan), as described previously (13). As primary antibodies, a mouse monoclonal anti-RNF126 antibody (sc-376005, 1:50 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and a mouse monoclonal anti-human Ki67 antibody (M7240, 1:100 dilution; DAKO) were employed. Antigen retrieval was carried out by heating tissue sections using a Target Retrieval Solution at pH 9.0 (DAKO). To block endogenous peroxidase, sections were immersed in a 3% solution of hydrogen peroxide in absolute methanol for 20 min at room temperature. To evaluate the RNF126 and Ki67 expression, ≥1,000 tumor cells from the invasive front of tumors were scored at per field ×400 magnification, and the percentage of tumor cells showing positive staining was calculated. A cutoff point for RNF126 was selected to give the optimal separation between low-risk and high-risk patients in terms of overall survival (OS).

*GC cell lines*. MKN45 (poorly differentiated adenocarcinoma) and MKN74 (moderately differentiated adenocarcinoma) human GC cell lines were purchased from the RIKEN BioResource Center and cultured in RPMI 1640 supplemented with 10% FBS.

*Real-time reverse transcriptase PCR*. Total RNA was isolated, and cDNA synthesis was carried out. The real-time reverse transcriptase PCR analysis was then performed, as described previously (13). The ratio of the mRNA level of RNF126 was calculated as follows: (absolute copy number of RNF126)/(absolute copy number of β2-microglobulin).

*Western blotting*. Cell lysates were prepared, and subjected to immunoblotting analysis using antibodies against RNF126 (ab183102, 1:250 dilution; Abcam, Burlingame, CA USA), β-actin (8H10D10, 1:1,000 dilution; Cell Signaling Technology, Danvers, MA, USA), p21 (sc-397, 1:100 dilution; Santa Cruz Biotechnology) and p27 (sc-528, 1:100 dilution; Santa Cruz Biotechnology).

*Small interfering RNA transfection of RNF126*. MKN45 and MKN74 cells were transfected with nonspecific control RNA (QIAGEN, Valencia, CA, USA) or 20 nmol/l of RNF126 siRNA (QIAGEN). Transfection was performed using the Lipofectamine system (Invitrogen), as described previously (13). The siRNA against RNF126 was selected to target the following DNA sequence: 5'-CCGGATTATATCTGTCCAAGA-3'.

*Cell viability assay and cell cycle analysis*. Cell viability was determined using the CellTiter-Blue® Cell Viability Assay kit (Promega Corp., Madison, WI, USA), as described previously (23). Briefly, aliquots of $5\times10^3$ of MKN45 and $3\times10^3$ of MKN74 cells per well were cultured in 96-well plates at 37˚C for 24 h, then the cells were transfected with control or RNF126 siRNA. Following 72 h of incubation at 37˚C, CellTiter-Blue® reagent was added to each well. Each experiment was performed at least three times. The cell-cycle analysis was performed using a Cycletest™ Plus DNA Reagent Kit (BD Biosciences, San Jose, CA, USA), as described previously (23).

*Statistical analysis*. Results were expressed as the means and standard deviations or as numbers and percentages, and Student's *t*-test, a one-way ANOVA, the Chi-squared test, or Fisher's exact test was used for evaluating statistical significance. OS was defined as the duration from surgery to death. Survival curves were estimated according to the Kaplan–Meier method, and the log-rank test was carried out to determine differences in survival rate. A Cox proportional hazards model was used for univariate and multivariate survival analyses. All significant variables in the univariate analysis were used in a multivariate analysis. A Pearson correlation analysis was performed to examine the correlation between the expression levels of RNF126 and Ki67. A *p*-value less than 0.05 was considered statistically significant and confidence intervals (CI) were calculated at the 95% level. All statistical analyses were performed using the SPSS® software program, version 19.0 (SPSS, Chicago, IL, USA).

## Results

In 161 (94.7%) GC tissue specimens, RNF126 was expressed in the cytoplasm of GC cells. The mean percentage of RNF126-positive cells in GC tissues was 43.5±26.7%.

We evaluated the relationships between RNF126 expression and clinicopathological characteristics. The positive rate of RNF126 was significantly associated with the tumor depth ($p$=0.001), pathological stage ($p$=0.008) and venous invasion ($p$=0.001; Table I).

The HR for high RNF126 for OS was highest when the cutoff value for the RNF126 expression was 63% (HR 2.865). Thus, the cutoff value for the RNF126 expression was set at 63%. Then, 51 patients (30%) with RNF126 positivity of ≥63% and 119 patients (70%) with RNF126 positivity of <63% were classified as the RNF126-high and RNF126-low groups, respectively (Figure 1a). The five-year OS rate of the RNF126-high group was significantly lower than that of the RNF126-low group (45.4% *vs*. 78.6%, $p$<0.001; Figure 1b). When comparing the survival rate according to tumor stage (Figure 1c, d and e), the RNF126-high group had a significantly lower OS rate than the RNF126-low group among patients with stage III disease (20.9% *vs*. 64.1%, $p$=0.002). The multivariate analysis showed that the RNF126 status, as well as tumor depth and lymph node metastasis, was an independent prognostic factor for OS ($p$<0.001; Table II).

At the time of analysis, 45 patients (26.5%) had postoperative recurrence. Overall, the recurrence rate was higher in RNF126-high tumors than in RNF126-low tumors (Table III). Hematogenous recurrence occurred more frequently in RNF126-high tumors than in RNF126-low tumors ($p$=0.008).

The involvement of RNF126 in the proliferation of GC cells was investigated, since RNF126 has been suggested to be required for the proliferation of cancer cells (14, 20, 24). A Ki67-positive rate was significantly higher in RNF126-high tumors than in RNF126-low tumors (28.4±12.8% *vs*. 19.4±11.6%, $p$<0.001; Figure 1f). Furthermore, we found a significant correlation between the expression levels of RNF126 and Ki67 ($p$<0.001, r=0.34).

Table I. *RNF126 expression and clinicopathological characteristics.*

| Variables | N | Percentage of RNF126-positive tumor cells | *p*-Value |
|---|---|---|---|
| Gender | | | |
| Male | 128 | 47.0±25.9 | 0.003[a] |
| Female | 42 | 32.9±26.7 | |
| Age (years) | | | |
| <67 | 78 | 39.3±24.4 | 0.060[a] |
| ≥67 | 92 | 47.0±28.2 | |
| Histology | | | |
| Differentiated | 94 | 45.9±28.3 | 0.196[a] |
| Undifferentiated | 76 | 40.5±24.5 | |
| Tumor size (mm) | | | |
| <50 | 111 | 41.1±27.0 | 0.113[a] |
| ≥50 | 59 | 48.0±25.9 | |
| Tumor depth | | | |
| T1 | 21 | 30.0±23.7 | 0.001[b] |
| T2 | 59 | 38.0±27.5 | |
| T3 | 54 | 46.9±25.0 | |
| T4 | 36 | 55.2±24.8 | |
| Lymph node metastasis | | | |
| Negative | 65 | 41.7±28.3 | 0.481[a] |
| Positive | 105 | 44.6±25.8 | |
| Pathological stage | | | |
| IB | 50 | 34.2±27.1 | |
| IIA, IIB | 67 | 45.2±25.7 | |
| IIIA, IIIB, IIIC | 53 | 50.1±26.7 | 0.008[b] |
| Lymphatic invasion | | | |
| Negative | 31 | 37.9±27.6 | 0.195[a] |
| Positive | 139 | 44.8±26.5 | |
| Venous invasion | | | |
| Negative | 99 | 38.0±27.2 | 0.001[a] |
| Positive | 71 | 51.1±24.2 | |

RNF126: Ring finger protein 126. Values are expressed as the mean and standard deviation. [a]Student's *t*-test. [b]One-way analysis of variance (ANOVA).

We further examined the effects of RNF126 down-regulation using an RNA interference method. When transfected with RNF126 siRNA for up to 72 h, both the mRNA and protein expression levels of RNF126 in human GC cell lines MKN45 and MKN74 were substantially reduced (Figure 2a and b). As a result, the cell proliferation was significantly inhibited by RNF126 gene silencing in these cells (Figure 2c).

To precisely investigate the effect of RNF126 knockdown, we performed cell-cycle analysis. The percentage of cells in the $G_1$ phase was significantly increased by RNF126 gene silencing in both cell lines [MKN45, control *vs*. RNF126 siRNA, 56.8±0.6% *vs*. 65.6±0.7% ($p<0.001$); MKN74, control *vs*. RNF126 siRNA, 48.5±0.1% *vs*. 56.5±0.1% ($p<0.001$); Figure 3a]. Western blotting showed that the protein level of p21 was increased by RNF126 knockdown (Figure 3b).

## Discussion

Recently, increasing attention has been paid to the involvement of dysfunction of the ubiquitin systems in cancer progression (23, 25, 26). In GC, several studies have investigated the expression of E3 ubiquitin ligases, such as Skp2, RBX1 and FBXW7, and reported that their expression levels were independently associated with survival (10-13). In the present study, we evaluated the RNF126 expression, an E3 ubiquitin ligase, and demonstrated its clinical importance in GC. Some researchers have investigated the prognostic impact of RNF126 in cancer patients. Yang *et al*. evaluated the expression of RNF126, and identified the high expression of RNF126 as an independent predictor of a poor prognosis in breast cancer patients (21). Wang *et al*. reported that the decreased expression of RNF126 was correlated with improved disease-free and OS in patients with ovarian serous cystadenocarcinoma (19). In the present study, RNF126 was found to be abundant in GC tissues, and the higher expression of RNF126 was significantly associated with poor OS. Importantly, the RNF126 expression was identified as an independent predictor of OS. Our studies suggest that RNF126 is a potential prognostic biomarker for GC.

It has been suggested that the aberrant expression of E3 ubiquitin ligases may have clinical relevance in cancer patients (11-13). However, there have been only limited reports on the expression of RNF126 in cancer tissue samples. Yang *et al*. evaluated the clinical significance of RNF126 in breast cancer, and reported no significant associations between the RNF126 expression level and clinicopathological factors such as tumor stage, histologic grade and molecular subtype in breast cancer (21). In this study, we found significant correlations between the RNF126 expression and tumor-related factors, including tumor depth, tumor stage and venous invasion. These findings indicate that RNF126 may contribute to the progression of GC.

In the present study, we also revealed that the overexpression of RNF126 was associated with a higher risk of GC recurrence. Yoshino *et al*. showed that the higher expression of RNF126 was associated with decreased distant metastasis-free survival in breast cancer patients (18). In the present study, the higher expression of RNF126 was significantly correlated with poor OS in patients with stage III disease. In addition, RNF126-high tumors were associated with a significantly higher rate of postoperative recurrence in comparison to RNF126-low tumors. In particular, hematogenous recurrence occurred more frequently in cases involving RNF126-high tumors, and this higher rate of hematogenous recurrence was partly explained by the significant association between the expression level of RNF126 and venous invasion. These data indicate that RNF126 may be a useful predictor of postoperative recurrence in GC.

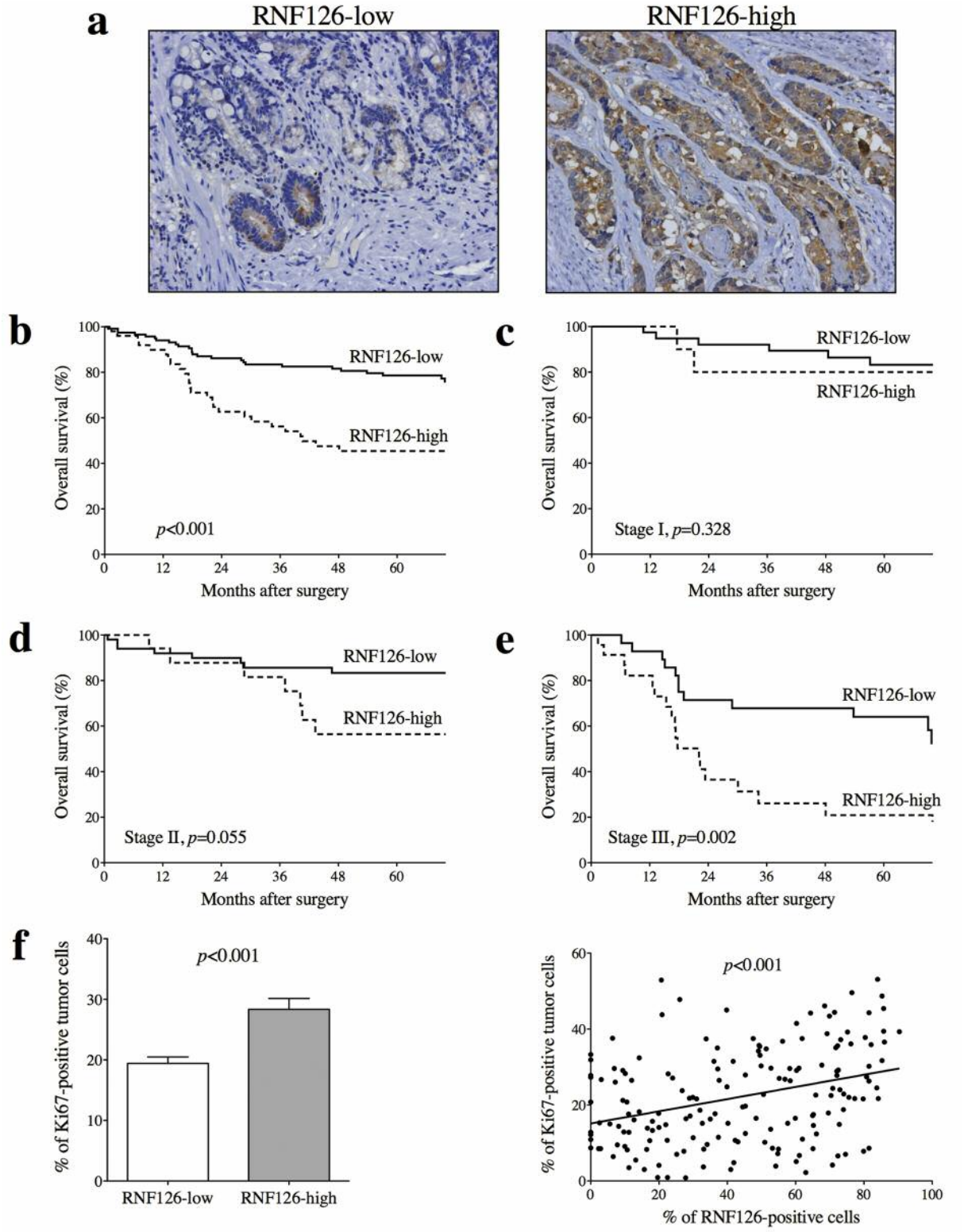


Figure 1. *RNF126 expression in gastric cancer. a: Representative cases of low and high expression of RNF126. Original magnification, ×200. b: Postoperative overall survival (OS) rate was significantly lower in patients with RNF126-high tumors than in patients with RNF126-low tumors (p<0.001). c-e: Kaplan-Meier estimates of OS according to the tumor stage (c stage I, d stage II, e stage III). f: A percentage of Ki67-positive tumor cells was significantly higher in RNF126-high tumors than in RNF126-low tumors (p<0.001). The RNF126 expression level was positively correlated with the Ki67 expression level (p<0.001, r=0.34).*

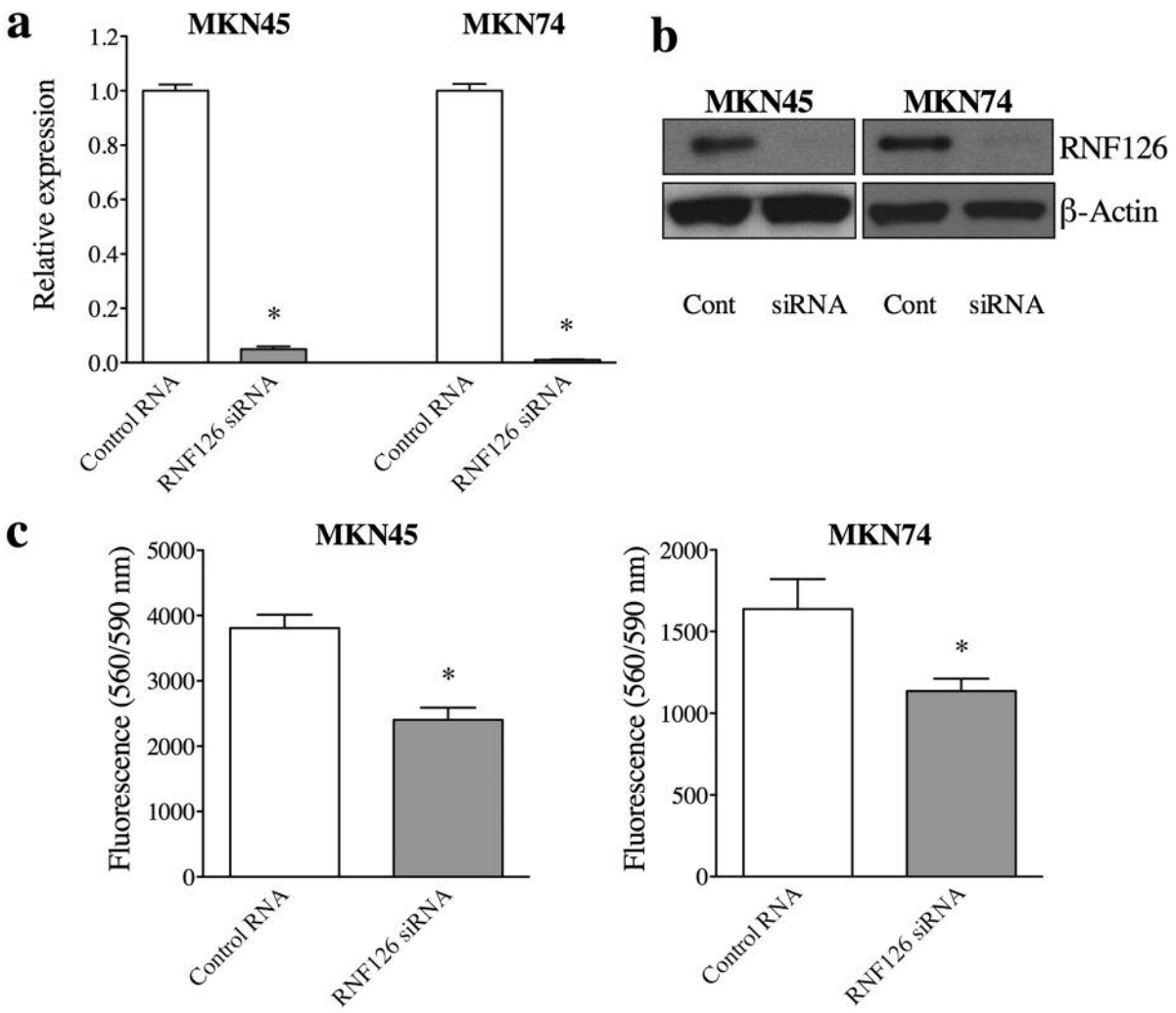


Figure 2. *RNF126 regulates the proliferation of gastric cancer cells. a: MKN45 and MKN74 cells were transfected with control RNA or RNF126 siRNA. RNF126 expression was evaluated by quantitative real-time PCR. The expression level of mRNA of RNF126 was reduced in both cell lines when transfected with RNF126 siRNA for up to 72 hours (n=3 of each group). b: Total protein lysates were extracted from treated cells and subjected to immunoblotting. The protein expression of RNF126 was also reduced in both cell lines. c: After 72 h incubation, cell proliferation was determined by the CellTiter-Blue® Cell Viability assay. The proliferation of cells treated with RNF126 siRNA was significantly inhibited in comparison to the control (n=10 of each group). *p<0.001.*

In addition, we evaluated the effect of the RNF126 expression level on the proliferation of GC cells. A number of studies have reported that dysfunction of E3 ubiquitin ligases contributes to stimulating cancer cell proliferation (9, 25, 27). Zhi *et al*. screened an E3 ubiquitin ligase siRNA library in breast cancer and prostate cancer cell lines, and identified RNF126 as a novel pro-proliferation target (14). The authors reported that knockdown of the RNF126 expression significantly inhibited cancer cell proliferation. The knockdown of the RNF126 expression was also reported to reduce the cell viability of tongue cancer cells and lung cancer cells (18, 20). In the present study, we found that a percentage of Ki67-positive tumor

Table II. *Results of the multivariate analysis of factors associated with survival.*

| Variables | HR | 95% CI | *p*-Value[a] |
|---|---|---|---|
| Tumor depth | | | |
| T3, T4/T1, T2 | 1.843 | 1.003-3.384 | 0.049 |
| Lymph node metastasis | | | |
| Positive/negative | 2.700 | 1.432-5.092 | 0.002 |
| Venous invasion | | | |
| Positive/negative | 1.282 | 0.713-2.308 | 0.407 |
| RNF126 expression | | | |
| High/low | 2.668 | 1.568-4.538 | <0.001 |

HR: Hazard ratio; CI: confidence interval; RNF126: ring finger protein 126. [a]Cox proportional hazards model.

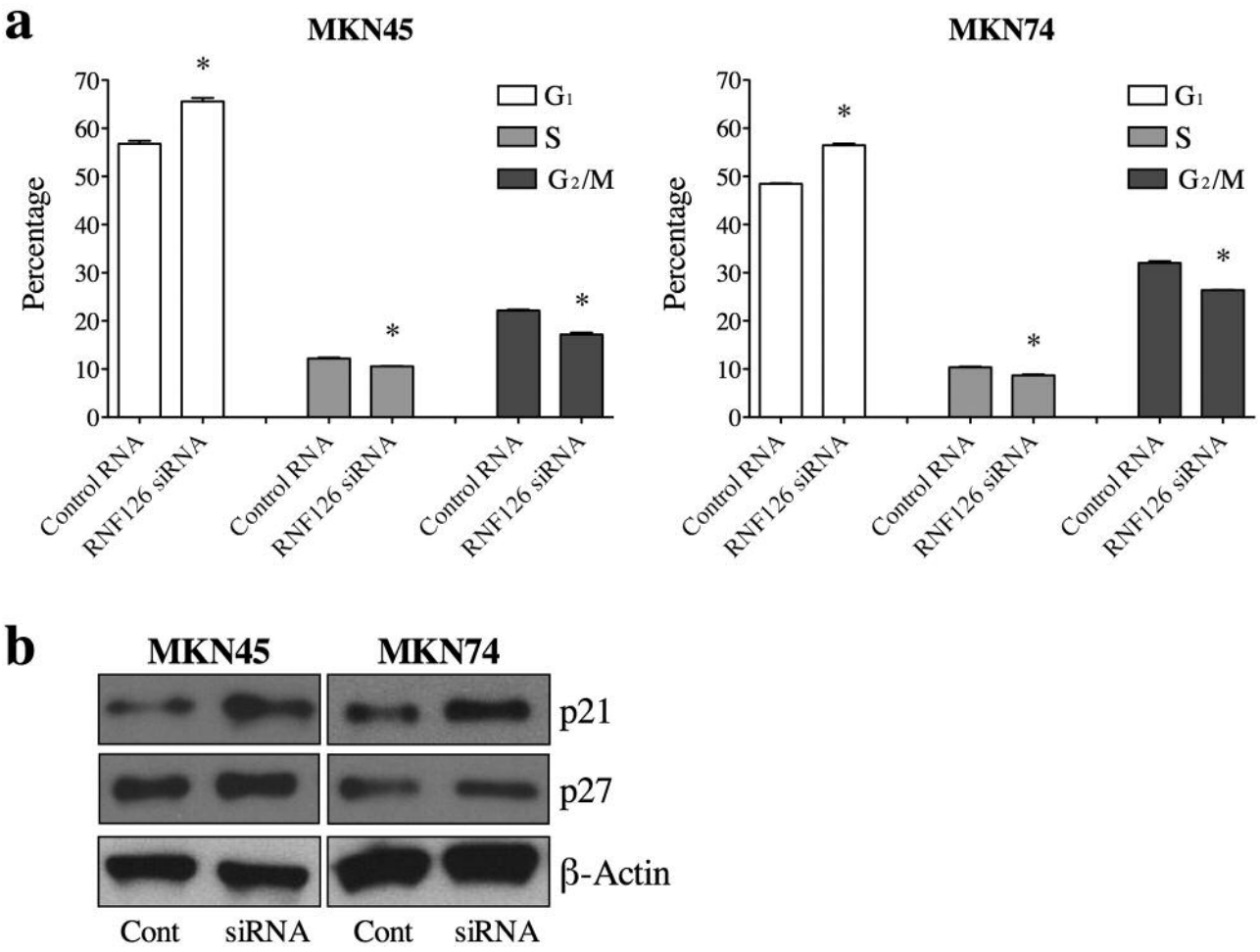


Figure 3. *Down-regulation of RNF126 induced $G_1$ phase arrest. a: MKN45 and MKN74 cells were transfected with control RNA or RNF126 siRNA, and then subjected to PI staining. The cell-cycle profile was analyzed by FACS. b: The protein levels of p21, p27 and β-actin were analyzed by Western blotting. *p<0.001.*

Table III. *Impact of the RNF126 status on postoperative recurrencea.*

| Site | RNF126-low (n=119, %) | RNF126-high (n=51, %) | OR | 95% CI | *p*-Value |
|---|---|---|---|---|---|
| All recurrence | 22 (18.5) | 23 (45.1) | 3.622 | 1.763-7.441 | <0.001[b] |
| Peritoneal | 13 (10.9) | 11 (21.6) | 2.242 | 0.929-5.414 | 0.068[b] |
| Hematogenous | 6 (5) | 9 (17.6) | 4.036 | 1.354-12.028 | 0.008[b] |
| Lymph nodal | 6 (5) | 4 (7.8) | 1.603 | 0.432-5.941 | 0.348[c] |

RNF126: Ring finger protein 126; OR: odds ratio; CI: confidence interval. [a]Some patients had a first recurrence at more than one site. [b]Chi-squared test. [c]Fisher's exact test.

cells was significantly higher in RNF126-high tumors than in RNF126-low tumors. Furthermore, a positive correlation between the expression levels of RNF126 and Ki67 was observed. In addition, we examined the association between the RNF126 expression level and the cell viability of GC cells using an RNA interference method *in vitro*, and observed that RNF126 gene silencing significantly reduced the cell proliferation. These findings suggest that RNF126 may play an important role in the proliferation of GC cells.

We further investigated the mechanisms underlying the inhibition of cell proliferation observed with RNF126 gene silencing. E3 ubiquitin ligases are well known to ubiquitinate and degrade various cell cycle regulators (9, 25, 27). Zhi *et al*. reported that RNF126 silencing delayed $G_1$-S progression and increased the protein level of cell cycle-dependent kinase inhibitor p21 in cancer cells (14). The authors also showed that the overexpression of RNF126 increased p21 protein ubiquitination. Consistent with these results, we showed that knockdown of the RNF126 expression resulted in $G_1$ cell cycle arrest and increased the p21 protein level. These findings suggested that RNF126 might partly promote cell cycle progression through regulation of the level of p21 in GC. However, further investigations are needed to elucidate the precise role of RNF126 in the progression of GC.

Given its overexpression and prognostic value, RNF126 may represent a potential target for cancer treatment. Zhi *et al*. demonstrated that RNF126 gene silencing significantly inhibited tumor growth *in vivo* (14). Other studies also showed that knockdown of the RNF126 expression suppressed tumor growth in mouse models (18, 20). Recently, MLN4924, a small-molecule inhibitor of the cullin-RING E3 ubiquitin ligases, was developed (28). MLN4924 is currently under investigation for cancer treatment in clinical trials (29, 30). Taken together, RNF126 could be an attractive therapeutic target for cancer.

In conclusion, the present study demonstrated that the RNF126 expression has significant prognostic value in GC. Furthermore, our study suggests that RNF126 may play an important role in regulating the proliferation of GC cells. The results of the present study may provide the rationale for developing novel treatments targeting RNF126 in GC.

## Conflicts of Interest

All Authors have no conflicts of interest to disclose.

## Authors' Contributions

This study was designed and directed by KM and MS. Data were collected and analyzed by KM, SM, KW, TK and HN. KM, TK and HN performed and analyzed immunohistochemistry. KM, TK and SM performed and analyzed *in vitro* studies. The article was written by KM and commented on by all Authors.

## Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP16K10513.

## References

1 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer *136(5)*: E359-386, 2015. PMID: 25220842. DOI: 10.1002/ijc.29210

2 Nashimoto A, Akazawa K, Isobe Y, Miyashiro I, Katai H, Kodera Y, Tsujitani S, Seto Y, Furukawa H, Oda I, Ono H, Tanabe S and Kaminishi M: Gastric cancer treated in 2002 in japan: 2009 annual report of the JGCA nationwide registry. Gastric Cancer *16(1)*: 1-27, 2013. PMID: 22729699. DOI: 10.1007/s10120-012-0163-4

3 Katai H, Ishikawa T, Akazawa K, Isobe Y, Miyashiro I, Oda I, Tsujitani S, Ono H, Tanabe S, Fukagawa T, Nunobe S, Kakeji Y and Nashimoto A: Five-year survival analysis of surgically resected gastric cancer cases in japan: A retrospective analysis of more than 100,000 patients from the nationwide registry of the Japanese Gastric Cancer Association (2001-2007). Gastric Cancer *21(1)*: 144-154, 2018. PMID: 2841726. DOI: 10.1007/s10120-017-0716-7

4 Nakamura K, Tomioku M, Nabeshima K and Nomura E: Clinical implication of the width of subserosal invasion in T3N0 gastric cancer. In Vivo *31(3)*: 409-413, 2017. PMID: 28438870. DOI: 10.21873/invivo.11074

5 Shitara K, Yodo Y and Iino S: A phase I study of napabucasin plus paclitaxel for japanese patients with advanced/recurrent gastric cancer. In Vivo *33(3)*: 933-937, 2019. PMID: 31028219. DOI: 10.21873/invivo.11561

6 Hershko A and Ciechanover A: The ubiquitin system. Annu Rev Biochem *67*: 425-479, 1998. PMID: 9759494. DOI: 10.1146/annurev.biochem.67.1.425

7 Ciechanover A and Iwai K: The ubiquitin system: From basic mechanisms to the patient bed. IUBMB Life *56(4)*: 193-201, 2004. PMID: 15230346. DOI: 10.1080/1521654042000223616

8 Nakayama KI and Nakayama K: Regulation of the cell cycle by SCF-type ubiquitin ligases. Semin Cell Dev Biol *16(3)*: 323-333, 2005. PMID: 15840441. DOI: 10.1016/j.semcdb.2005.02.010

9 Frescas D and Pagano M: Deregulated proteolysis by the f-box proteins SKP2 and beta-TrCP: Tipping the scales of cancer. Nat Rev Cancer *8(6)*: 438-449, 2008. PMID: 18500245. DOI: 10.1038/nrc2396

10 Masuda TA, Inoue H, Sonoda H, Mine S, Yoshikawa Y, Nakayama K, Nakayama K and Mori M: Clinical and biological significance of S-phase kinase-associated protein 2 (Skp2) gene expression in gastric carcinoma: Modulation of malignant phenotype by Skp2 overexpression, possibly *via* p27 proteolysis. Cancer Res *62(13)*: 3819-3825, 2002. PMID: 12097295.

11 Yokobori T, Mimori K, Iwatsuki M, Ishii H, Onoyama I, Fukagawa T, Kuwano H, Nakayama KI and Mori M: p53-altered FBXW7 expression determines poor prognosis in gastric cancer cases. Cancer Res *69(9)*: 3788-3794, 2009. PMID: 19366810. DOI: 10.1158/0008-5472.Can-08-2846

12 Bai J, Zhou Y, Chen G, Zeng J, Ding J, Tan Y, Zhou J and Li G: Overexpression of Cullin1 is associated with poor prognosis of patients with gastric cancer. Hum Pathol *42(3)*: 375-383, 2011. PMID: 21190721. DOI: 10.1016/j.humpath.2010.09.003

13 Migita K, Takayama T, Matsumoto S, Wakatsuki K, Tanaka T, Ito M, Nishiwada S and Nakajima Y: Prognostic impact of RING box protein-1 (RBX1) expression in gastric cancer. Gastric Cancer *17(4)*: 601-609, 2014. PMID: 24292229. DOI: 10.1007/s10120-013-0318-y

14 Zhi X, Zhao D, Wang Z, Zhou Z, Wang C, Chen W, Liu R and Chen C: E3 ubiquitin ligase RNF126 promotes cancer cell proliferation by targeting the tumor suppressor p21 for ubiquitin-

mediated degradation. Cancer Res *73(1)*: 385-394, 2013. PMID: 23026136. DOI: 10.1158/0008-5472.Can-12-0562

15 Smith CJ, Berry DM and McGlade CJ: The E3 ubiquitin ligases RNF126 and Rabring7 regulate endosomal sorting of the epidermal growth factor receptor. J Cell Sci *126(Pt 6)*: 1366-1380, 2013. PMID: 23418353. DOI: 10.1242/jcs.116129

16 Rodrigo-Brenni MC, Gutierrez E and Hegde RS: Cytosolic quality control of mislocalized proteins requires RNF126 recruitment to Bag6. Mol Cell *55(2)*: 227-237, 2014. PMID: 24981174. P DOI: 10.1016/j.molcel.2014.05.025

17 Ishida N, Nakagawa T, Iemura SI, Yasui A, Shima H, Katoh Y, Nagasawa Y, Natsume T, Igarashi K and Nakayama K: Ubiquitylation of Ku80 by RNF126 promotes completion of nonhomologous end joining-mediated DNA repair. Mol Cell Biol 37(4), 2017. PMID: 27895153. DOI: 10.1128/mcb.00347-16

18 Yoshino S, Hara T, Nakaoka HJ, Kanamori A, Murakami Y, Seiki M and Sakamoto T: The ERK signaling target RNF126 regulates anoikis resistance in cancer cells by changing the mitochondrial metabolic flux. Cell Discov *2*: 16019, 2016. PMID: 27462466. DOI: 10.1038/celldisc.2016.19

19 Wang Y, Deng O, Feng Z, Du Z, Xiong X, Lai J, Yang X, Xu M, Wang H, Taylor D, Yan C, Chen C, Difeo A, Ma Z and Zhang J: RNF126 promotes homologous recombination *via* regulation of E2F1-mediated BRCA1 expression. Oncogene *35(11)*: 1363-1372, 2016. PMID: 26234677. DOI: 10.1038/onc.2015.198

20 Wang L, Wang X, Zhao Y, Niu W, Ma G, Yin W and Shi C: E3 ubiquitin ligase RNF126 regulates the progression of tongue cancer. Cancer Med *5(8)*: 2043-2047, 2016. PMID: 27227488. DOI: 10.1002/cam4.771

21 Yang X, Pan Y, Qiu Z, Du Z, Zhang Y, Fa P, Gorityala S, Ma S, Li S, Chen C, Wang H, Xu Y, Yan C, Ruth K, Ma Z and Zhang J: RNF126 as a biomarker of a poor prognosis in invasive breast cancer and CHEK1 inhibitor efficacy in breast cancer cells. Clin Cancer Res *24(7)*: 1629-1643, 2018. PMID: 29326282. DOI: 10.1158/1078-0432.Ccr-17-2242

22 Sobin L, Gospodarowicz M and Wittekind C: TNM classification of mialignant tumors. 7th edn. Wiley-Blackwell: Oxford, 2010.

23 Nakade H, Migita K, Matsumoto S, Wakatsuki K, Kunishige T, Miyao S and Sho M: Overexpression of Cullin4a correlates with a poor prognosis and tumor progression in esophageal squamous cell carcinoma. Int J Clin Oncol, 2019. PMID: 31535245. DOI: 10.1007/s10147-019-01547-2

24 Yoshino S, Hara T, Weng JS, Takahashi Y, Seiki M and Sakamoto T: Genetic screening of new genes responsible for cellular adaptation to hypoxia using a genome-wide shRNA library. PLoS One *7(4)*: e35590, 2012. PMID: 22523603. DOI: 10.1371/journal.pone.0035590

25 Jia L, Soengas MS and Sun Y: ROC1/RBX1 E3 ubiquitin ligase silencing suppresses tumor cell growth *via* sequential induction of $G_2$-M arrest, apoptosis, and senescence. Cancer Res *69(12)*: 4974-4982, 2009. PMID:19509229. DOI: 10.1158/0008-5472.Can-08-4671

26 Michail O, Moris D, Theocharis S and Griniatsos J: Cullin-1 and -2 protein expression in colorectal cancer: Correlation with clinicopathological variables. In Vivo *32(2)*: 391-396, 2018. PMID: 29475926. DOI: 10.21873/invivo.11251

27 Schlabach MR, Luo J, Solimini NL, Hu G, Xu Q, Li MZ, Zhao Z, Smogorzewska A, Sowa ME, Ang XL, Westbrook TF, Liang AC, Chang K, Hackett JA, Harper JW, Hannon GJ and Elledge SJ: Cancer proliferation gene discovery through functional genomics. Science *319(5863)*: 620-624, 2008. PMID: 18239126. DOI: 10.1126/science.1149200

28 Soucy TA, Smith PG, Milhollen MA, Berger AJ, Gavin JM, Adhikari S, Brownell JE, Burke KE, Cardin DP, Critchley S, Cullis CA, Doucette A, Garnsey JJ, Gaulin JL, Gershman RE, Lublinsky AR, McDonald A, Mizutani H, Narayanan U, Olhava EJ, Peluso S, Rezaei M, Sintchak MD, Talreja T, Thomas MP, Traore T, Vyskocil S, Weatherhead GS, Yu J, Zhang J, Dick LR, Claiborne CF, Rolfe M, Bolen JB and Langston SP: An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. Nature *458(7239)*: 732-736, 2009. PMID: 19360080. DOI: 10.1038/nature07884

29 Sarantopoulos J, Shapiro GI, Cohen RB, Clark JW, Kauh JS, Weiss GJ, Cleary JM, Mahalingam D, Pickard MD, Faessel HM, Berger AJ, Burke K, Mulligan G, Dezube BJ and Harvey RD: Phase I study of the investigational NEDD8-activating enzyme inhibitor pevonedistat (TAK-924/MLN4924) in patients with advanced solid tumors. Clin Cancer Res *22(4)*: 847-857, 2016. PMID: 26423795. DOI: 10.1158/1078-0432.Ccr-15-1338

30 Swords RT, Coutre S, Maris MB, Zeidner JF, Foran JM, Cruz J, Erba HP, Berdeja JG, Tam W, Vardhanabhuti S, Pawlikowska-Dobler I, Faessel HM, Dash AB, Sedarati F, Dezube BJ, Faller DV and Savona MR: Pevonedistat, a first-in-class NEDD8-activating enzyme inhibitor, combined with azacitidine in patients with AML. Blood *131(13)*: 1415-1424, 2018. PMID: 29348128. DOI: 10.1182/blood-2017-09-805895

*Received January 15, 2020*
*Revised January 25, 2020*
*Accepted January 28, 2020*