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# RING finger protein 187 as a novel potential biomarker for predicting the prognosis of ovarian carcinoma in 2 cancer centers

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## ABSTRACT

RING finger protein 187 (RNF187) has been used to predict prognosis of several human carcinomas. However, the clinicopathologic and prognostic implication of RNF187 expression in ovarian carcinomas remains not to be evaluated. The aim of this study was to explore the clinicopathologic and the prognostic significance of RNF187 in patients with ovarian carcinomas. Expression levels of RNF187 protein were investigated by immunohistochemical staining based on tissue-microarray composed of 147 patients with ovarian carcinomas. Receiver operating characteristic curve analysis was used to select the ideal cut-off value of RNF187 expression in ovarian carcinoma, and then analyze the correlation between the status of RNF187 expression and various clinicopathologic variables by chi-square test. Univariate analysis was employed to investigate the association between clinicopathologic variables and prognosis of patients by Kaplan-Meier method. Multivariate analysis was performed to identify the independent prognostic factors by the Cox regression model. Our results demonstrated that high expression of RNF187 was significantly associated with late FIGO stage, high histologic grade and pN1 stage in ovarian carcinoma ($P < 0.05$). Univariate analysis

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uncovered patients with the high expression of RNF187 have the worse overall survival and disease-free survival ($P < 0.05$). More surprisingly, multivariate analysis determined that the RNF187 expression was served as an independent prognostic factor in ovarian carcinoma. The high expression of RNF187 might influence a more aggressive biological behavior in ovarian carcinoma. Therefore, RNF187 expression could be useful to act as a new independent prognostic biomarker for patients with ovarian carcinoma.



ARTICLE INFO

*Keywords:* RING finger protein 187 (RNF187); Ovarian carcinoma; Prognostic

## Introduction

Ovarian carcinoma is the fifth common cause of cancer-associated death for women with a mortality of approximately 54% in developed countries.[1] Increasing incidence and mortality rate of ovarian carcinoma have seriously affected health of Chinese women during the past decades.[2] Early metastasis of ovarian cancer in the process of progression results in the relentless mortality rate.[3] The diagnosis of a number of patients with ovarian cancer belongs to the late clinical stage. Meanwhile, although the multimodal therapeutic strategies were applied in clinical practice including cytoreductive surgery and intensive chemotherapy, and eventually, the 5-year survival rate of patients remains to be, at best, only 30% due to tumor recurrence.[4,5] The reason why occurred recurrence is that tumor acquired resistance to chemotherapeutic drugs. It is urgently necessary to find the potential mechanisms that lead to drug resistance associated with molecular and biological changes of ovarian cancer to improve the efficacy of treatment regime, and further acquired new prognostic biomarkers to more precisely predict prognosis of patients, which could be benefit for individual treatments for patients with ovarian cancer. Thus, it is urgent to explore the novel biomarkers for predicting prognosis, and investigate new effective therapeutic target for ovarian cancer.

Ubiquitin-proteasome system played a critical role on post-translational modifications and degradation of targeted protein.[6] Ubiquitin-protein ligases (E3s) determined the substrate specificity and conjugated distinct topologies in the process of ubiquitination.[7,8] The ubiquitin-proteasome system has recently emerged as a pivotal player in controlling the cellular process including homeostasis, apoptosis, cell cycle, metabolism, and immune responses. However, aberrant ubiquitylation often leads to birth defects, diseases, or tumorigenisis.[7-9] RING finger E3s have different function for the transformation of the malignancy, which are served as either oncogenes or tumor suppressor genes owing to conjugating with variable substrates or different roles of a single substrate.[9] Ring finger protein 4 (RNF4s) as a member of monomeric RING domain E3 ligase family are elevated expression in colon adenocarcinoma and breast cancer cell lines, and patients with high expression have the worse prognosis than that with low expression in colon adenocarcinoma, suggesting a potential tumorigenic role of RNF4s in colon adenocarcinoma.[10] A study reported that RING finger protein 187 (RNF187 or RACO1), as a another member of the family including a RING domain-containing E3, was overexpression in patients with hepatocellular carcinoma, which has the poor prognosis than that with low expression of RNF187,[11] and RNF187 was recently showed that increased expression in non–small cell carcinoma (NSCLC) and promote NSCLC progression by inducing cell apoptosis resistance and (Epithelial-Mesenchymal Transition) EMT.[12] Mechanism study showed RNF187 is considered as the coactivator of c–Jun independently of aminoterminal phosphorylation, and is necessary and sufficient for activation of c-Jun/AP-1, which process is also involved in the Arginine methylation of RNF187 enables it stable that is important for linking to Jun-1[13,14] and RNF187 depletion

resulted in the reduction in cellular proliferation, delayed cell-cycle re-entry, and downregulation of several growth-related AP-1 target genes, including cdc2 and cyclinD1.[13] cDNA array analysis demonstrated that the level of RNF187 was downregulated in response to NF-$\kappa$B inhibition, which signal pathway as a pivotal player in multiple steps of cancer initiation and progression.[15,16] However, there are no relevant studies on the prognostic value of RNF187 in ovarian carcinoma. Therefore, the aim of this study is to elucidate the status of RNF187 expression by tissue-microarray-based immunohistochemistry (IHC) and its prognostic significance in ovarian carcinoma.

## Materials and methods

### *Ethics statement*

The study was supported by the Institute Research Medical Ethics Committee of Sun Yat-sen University and Jiangmen Central Hospital. In this retrospective study, no written or verbal consent was achieved for patients' tissue samples with ovarian carcinoma. The majority of these patients were deceased, who waived the need for consent, thus for the ethics committee, this is unnecessary. All samples were anonymized.

### *Patients and tissue specimens*

We collected paraffin tissue samples of ovarian carcinoma from 2 medical centers, including 75 cases were collected in Sun Yat-sen University Cancer Center and 72 cases came from Jiangmen Central Hospital of Guangdong province between October 2003 and September 2014. In this study, the diagnostic criterion of all tissue samples was based on the 2014 WHO criteria for tumor classification, and tumor staging was on the basis of International Federation Gynecology and Obstetrics (FIGO), and tumors were graded according to the Silverberg grading system.

### *IHC*

RNF187 protein was stained by IHC according to standard EnVision procedure. We sliced the paraffin blocks into 3-$\mu$m sections and dry sections in incubator at 60°C. Slides were deparaffinized with xylene and rehydrated by graded ethanol series, and then immersed in 3% hydrogen peroxide for 10 minutes to inhibit the endogenous peroxidase activity, subsequently placed in boiled citric acid buffer for antigen retrieval. We incubated the slides with antibody RNF187 (Novus, rabbit polyclonal, operative solution concentration 1:50) at 37°C for 50 minutes in the incubator. Hereafter, they were incubated with secondary antibody (DAKO, K5007) at 37°C for 30 minutes in the incubator. Then, the staining was applied with 3, 3-diaminobenzidine and the degree of staining was observed by microscope. Finally, the slides were counterstained with hematoxylin, dehydrated in a graded ethanol series, cleared in xylene, and mounted with neutral gum. Positive and negative controls were set in the staining procedure.

### *IHC evaluation*

The status of RNF187 expression was assessed according to the method mentioned below. The presence of cytoplasmic dark brown was identified to be positive for RNF187 expression, and scoring criteria as follows: each sample tissue harbored an intensity score (I score) from 0 to 3 (such as I0, I1, I2, and I3: I0 equals to negative expression, I1 equals to weak expression, I2 equals to moderate expression, and I3 equals to strong expression). Subsequently,

RNF187 was elucidated according to the percentage of positively stained cells in 5% increments ranging from 0% to 100%, which obtained a percentage score (P score). The total H score (range from 0 to 300) was calculated by multiplying each I score and P score, such as H score = I1 × P1 + I2 × P2 + I3 × P3.

### *Selection of cut-off value*

The plot of TPF (sensitivity) vs FPF (1-specificity) by various cutoffs generates a curve that called an receiver operating characteristic (ROC) curve in the unit square, ROC curve analysis could determine the optimal cut-off value by the point (0.0, 1.0) or (1.0, 0.0).[17,18] The sensitivity and specificity for every clinicopathologic variable were plotted in the study, and performing the corresponding ROC curves for the RNF187 score. The score was selected as the cut-off value which was closest to the point with both maximum sensitivity and specificity. The score less than or equal to the cut-off value was served as low expression of RNF187, and more than the cut-off value was identified as high expression. The clinicopathologic characteristics were involved in the ROC curve analysis, including FIGO stage, pN status, histologic type, tumor recurrence, survival status, and the level of serum CA199.

### *Statistical analysis*

Statistical analyses were performed using SPSS software, version 16.0 (SPSS, Chicago, IL). The correlation between RNF187 expression and clinicopathologic variables of patients with ovarian carcinoma was analyzed by chi-square test. Univariate analysis was used to determine the association between clinicopathologic variables and overall survival and disease-free-survival of patients by Kaplan-Meier method. Multivariate analysis was performed by the Cox regression model to identify the independent prognostic factors. A 2-tailed *P* value less than 0.05 was served as statistically significant in all cases.

## Results

### *Patients' characteristics*

The clinicopathologic features of patients with ovarian carcinoma are demonstrated in Table 1. One hundred forty-seven patients with ovarian carcinoma enrolled in our study showed mean age of 49.0 years. The follow-up period ranges from 4.0 to 159.0 months (median, 68.3 months). Eighty-nine patients (60.5%) with late stages (FIGO III and IV) were diagnosed, and other 58 patients (39.5%) belong to early stages (FIGO I and II).

### *Choice of the cut-off score for RNF187 expression*

The status of expression of RNF187 protein in tissues with ovarian carcinoma was displayed in Fig. 1. To choose a suitable cut-off score of RNF187 for further analysis, there is a point in the ROC curves of each clinicopathologic characteristics closest to the point (0.0, 1.0) or (1.0, 0.0), which makes both the sensitivity and specificity maximize for the result. Tumor tissues with score more than the obtained cut-off value were identified as high expression of RNF187. As it was shown in Fig. 2, the survival status had the closest to the point (0.0, 1.0). On the basis of this outcome, we choose 170 score as cut-off value of RNF187 expression by the ROC curve of the survival status for survival analysis. Finally, our study showed that low expression of RNF187 could be found in 83 of 147 (56.5%) patients with ovarian carcinoma, and 64 of 147 (43.5%) patients with high expression.

**Table 1**
Correlation between the clinicopathologic variables and the expression of RNF187 in ovarian carcinoma.

| Variables | RNF187 expression | | | |
|---|---|---|---|---|
| | All cases | Low expression | High expression | *P* Value |
| Age (y) | | | | 0.654 |
| ≤49 | 75 | 41 (54.7%) | 34 (45.3%) | |
| >49 | 72 | 42 (58.3%) | 30 (41.7%) | |
| Histologic type | | | | 0.205 |
| Serous | 95 | 50 (52.6%) | 45 (47.4%) | |
| Mucinous | 52 | 33 (63.5%) | 19 (36.5%) | |
| FIGO stage | | | | **0.005** |
| I + II | 58 | 41 (70.7%) | 17 (29.3%) | |
| III + IV | 89 | 42 (47.2%) | 47 (52.8%) | |
| Pathologic grade | | | | 0.027 |
| G1 | 26 | 20 (76.9%) | 6 (23.1%) | |
| G2 | 45 | 27 (60.0%) | 18 (40.0%) | |
| G3 | 76 | 36 (47.4%) | 40 (52.6%) | |
| pN status | | | | **0.007** |
| pN0 | 62 | 43 (69.4%) | 19 (30.6%) | |
| pN1 | 85 | 40 (47.1%) | 45 (52.9%) | |
| Tumor size (cm) | | | | 0.874 |
| ≤10.6 | 77 | 43 (55.8%) | 34 (44.2%) | |
| >10.6 | 70 | 40 (57.1%) | 30 (42.9%) | |
| Serum CA125 | | | | 0.212 |
| ≤33 | 17 | 12 (70.6%) | 5 (29.4%) | |
| >33 | 130 | 71 (54.6%) | 59 (45.4%) | |
| Serum CA19-9 | | | | 0.094 |
| ≤35 | 97 | 50 (51.5%) | 47 (48.5%) | |
| >35 | 50 | 33 (66.0%) | 17 (34.0%) | |
| Serum CEA | | | | 0.353 |
| ≤5 | 119 | 65 (54.6%) | 54 (45.4%) | |
| >5 | 28 | 18 (64.3%) | 10 (35.7%) | |
| Tumor recurrence | | | | 0.121 |
| No | 93 | 57 (61.3%) | 36 (38.7%) | |
| Yes | 54 | 26 (48.1%) | 28 (51.9%) | |

FIGO stage: postoperation FIGO stage based on pathologic situation.

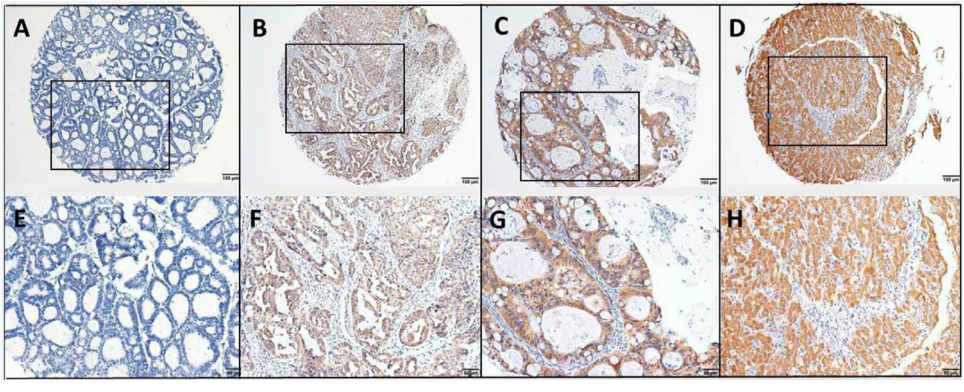


**Fig. 1.** Expression of RNF187 protein in ovarian carcinoma tissues. A (×10), E (×20): negative expression; B (×10), F (×20): low expression; C (×10), G (×20): moderate expression; D (×10), H (×20): strong expression.

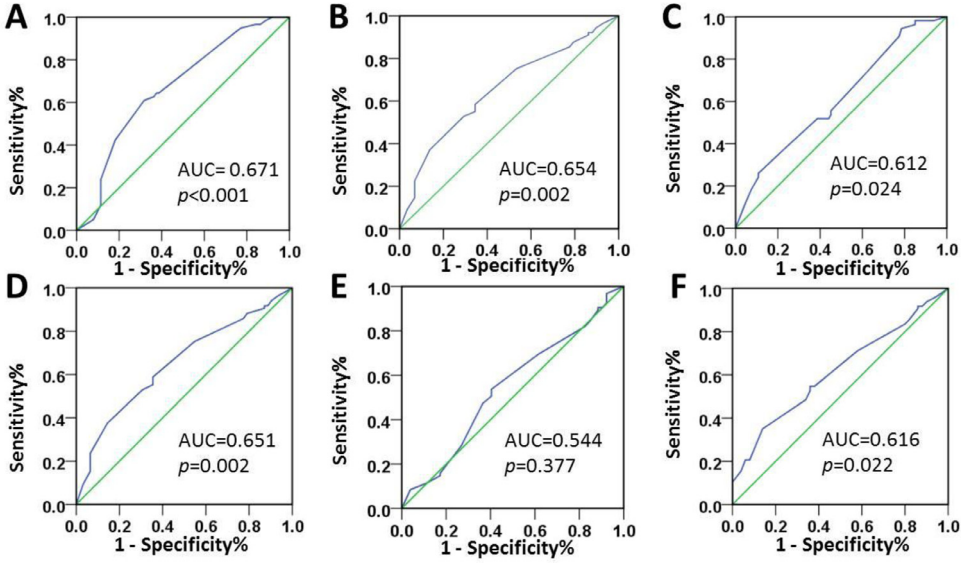


**Fig. 2.** ROC curve analysis was employed to determine the cut-off value for RNF187 expression in ovarian carcinoma. The sensitivity and specificity for each outcome were plotted: survival status (A), FIGO stage (B), tumor recurrence (C), pN status (D), histologic type (E), serum CA199 (F).

*The relationship between RNF187 expression and the clinicopathologic variables of patients with ovarian carcinoma*

The low and high expression rate of RNF187 in patients with ovarian carcinoma linked to several clinicopathologic variables are described in Table 1. The data showed that high expression of RNF187 was significantly correlated with late FIGO stage, high histologic grade and pN1 stage ($P < 0.05$, Table 1) and no significant association is shown between RNF187 expression and other clinicopathologic variables, including histologic type, tumor size, and tumor recurrence etc. ($P > 0.05$, Table 1).

*The relationship between RNF187 expression and patients' survival with ovarian carcinoma*

Univariate analysis revealed a significant impact of well-known clinicopathologic prognostic factors on patients' survival with ovarian carcinoma such as FIGO stage, pathologic grade, pN1 stage, and tumor recurrence ($P < 0.05$, Table 2). Univariate analysis also demonstrated that high expression of RNF187 was significantly associated with more adverse overall survival ($P < 0.001$, Fig 3A) and disease-free survival ($P = 0.009$, Fig 3B), and stratification analysis identified that high expression of RNF187 was intensively correlated with pessimistic overall survival in patients of ovarian carcinoma with histologic type of serous, histologic type of mucinous, late FIGO stage and pN1 stage, respectively ($P < 0.05$, Fig 4A,B,D,F). Moreover, high expression of RNF187 was closely correlated with poor disease-free survival in patients of ovarian carcinoma with histologic type of serous and pN1 stage, respectively ($P < 0.05$, Fig 5A and F). Cox regression model demonstrated that the high expression of RNF187 was an independent prognostic factor for overall survival and disease-free survival (hazard ratio: 2.053, 95% confidence interval: 1.194-3.529, $P = 0.009$; hazard ratio: 1.951, 95% confidence interval: 1.107-3.437, $P = 0.021$; Table 3).

**Table 2**
Univariate analysis of clinicopathologic variables in 147 patients with ovarian carcinoma (log-rank test).

| Variables | All cases | Mean survival (mo) | $\chi 2$ | *P* Value |
|---|---|---|---|---|
| Age (y) | | | 3.92 | **0.048** |
| ≤49 | 75 | 117.98 | | |
| >49 | 72 | 91.07 | | |
| Histologic type | | | 0.43 | 0.512 |
| Serous | 95 | 98.83 | | |
| Mucinous | 52 | 112.36 | | |
| FIGO stage | | | 42.6 | **<0.001** |
| I + II | 58 | 150.29 | | |
| III + IV | 89 | 74.22 | | |
| Pathologic grade | | | 18.01 | **<0.001** |
| G1 | 26 | 142.65 | | |
| G2 | 45 | 115.34 | | |
| G3 | 76 | 74.95 | | |
| pN status | | | 44.95 | **<0.001** |
| pN0 | 62 | 148.5 | | |
| pN1 | 85 | 72.46 | | |
| Tumor size (cm) | | | 0.34 | 0.560 |
| ≤10.6 | 77 | 104.86 | | |
| >10.6 | 70 | 92.54 | | |
| Serum CA125 | | | 7.54 | **0.006** |
| ≤33 | 17 | 119.82 | | |
| >33 | 130 | 102.13 | | |
| Serum CA19-9 | | | 1.65 | 0.199 |
| ≤35 | 97 | 102.88 | | |
| >35 | 50 | 97.49 | | |
| Serum CEA | | | 0.98 | 0.323 |
| ≤5 | 119 | 110.21 | | |
| >5 | 28 | 91.86 | | |
| Tumor recurrence | | | 12.93 | **<0.001** |
| No | 93 | 121.76 | | |
| Yes | 54 | 73.77 | | |
| RNF187 | | | 14.09 | **<0.001** |
| Low | 83 | 124.2 | | |
| High | 64 | 71.63 | | |

FIGO stage: postoperation FIGO stage based on pathologic situation.

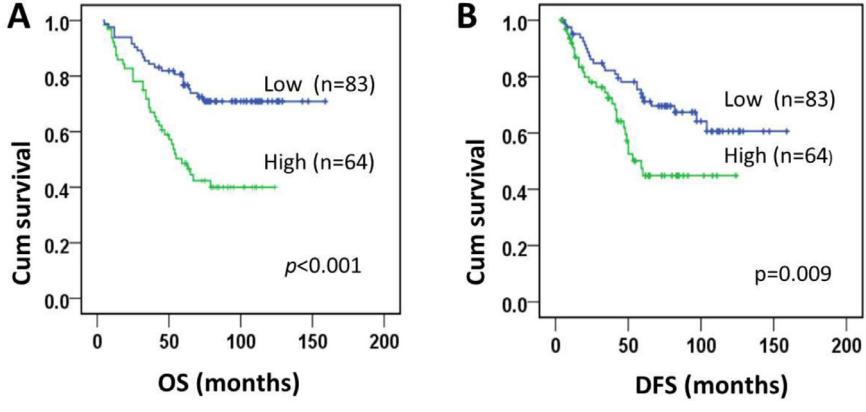


**Fig. 3.** Kaplan-Meier survival analysis of RNF187 expression in total patients with ovarian carcinoma. A: (OS), B: (DFS).

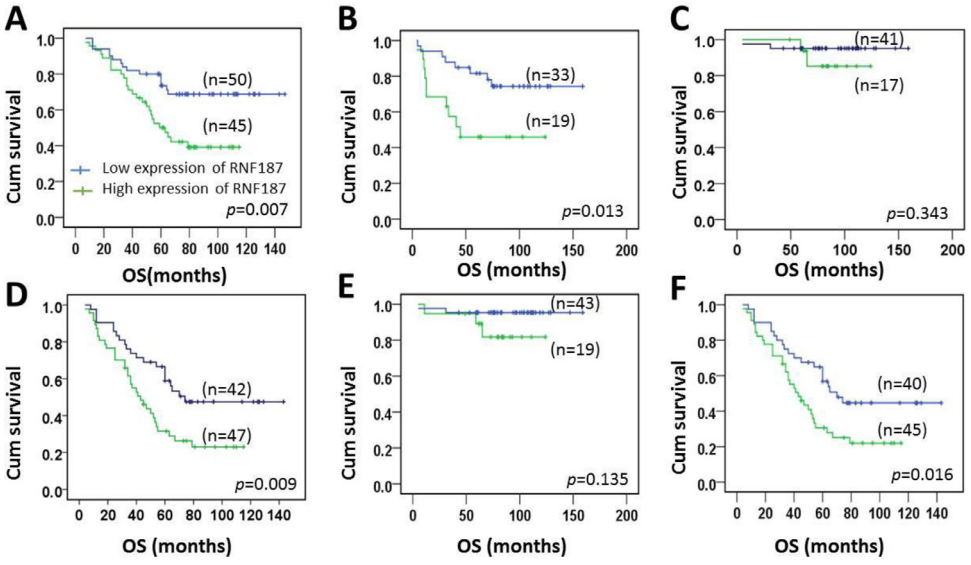


**Fig. 4.** Kaplan-Meier survival analysis of RNF187 expression in total patients and subsets of different variable with patients with ovarian carcinoma for overall survival. Histologic type, serous (A); histologic type, mucinous (B); FIGO, I+II (C); FIGO, III+IV (D); pN0 stage (E); pN1 stage (F).

**Table 3**
Multivariate survival analyses of clinicopathologic variables in patients with ovarian carcinoma.

| Variables | OS | | | PFS | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* Value | HR | 95% CI | *P* Value |
| FIGO (III + IV vs I + II) | 10.013 | 3.382-29.649 | **<0.001** | 4.113 | 1.952-8.668 | **<0.001** |
| Histologic type (serous vs mucinous) | 1.642 | 0.905-2.980 | 0.103 | 1.472 | 0.794-2.729 | 0.219 |
| Pathologic grade (G3 vs G1 + G2) | 1.786 | 0.950-3.357 | 0.072 | 1.306 | 0.710-2.404 | 0.391 |
| Tumor size (cm) (>10.6 vs ≤10.6) | 0.999 | 0.570-1.752 | 0.998 | 0.639 | 0.350-1.167 | 0.145 |
| Serum CA125 (>33 vs ≤33) | 1.656 | 0.202-13.582 | 0.639 | 3.748 | 0.479-29.301 | 0.208 |
| Serum CA19-9 (>35 vs ≤35) | 1.009 | 0.545-1.867 | 0.977 | 1.081 | 0.581-2.012 | 0.806 |
| Serum CEA (>5 vs ≤5) | 1.449 | 0.758-2.773 | 0.262 | 1.745 | 0.890-3.421 | 0.105 |
| RNF187 (high vs low) | 2.053 | 1.194-3.529 | **0.009** | 1.951 | 1.107-3.437 | **0.021** |

FIGO stage: postoperation FIGO stage based on pathologic situation.

## Discussion

Ovarian carcinoma has the highest mortality rate and the worst clinical outcome in female malignant epithelial tumors,[19] and is characterized by clinically occult dissemination and metastasis through exfoliating into the peritoneal cavity from primary ovarian tumor by normal peritoneal fluid flow compared with the blood route metastasis in other carcinomas.[20] Local and disseminated cells to chemoresistance remain to be the root of recurrence and distant metastasis despite in combination with surgery and chemotherapies due to the unique mode of dissemination.[21,22] FIGO stage is now being used worldwide to predict the prognosis of patients with ovarian carcinoma. Moreover, patients harboring same clinical stage could have different clinical outcome after receiving one identical therapeutic regime. Therefore, it is urgently needed to identify novel therapeutic strategies that might be beneficial to distinguish the prognosis of patients with the similar grade or clinical stage. Although prior studies have showed that several aberrantly alerted genes in ovarian carcinoma,[23,24] it is still necessary to explore new molecular biomarkers to predict tumor recurrence and risk assessment.

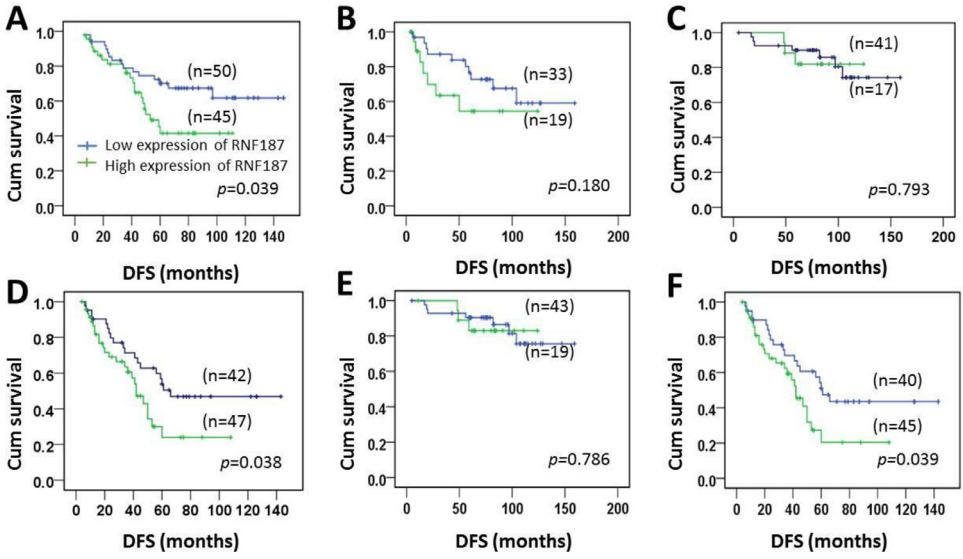


**Fig. 5.** Kaplan-Meier survival analysis of RNF187 expression in total patients and subsets of different variable patients with ovarian carcinoma for DFS. Histological type, Serous (A); Histological type, Mucinous (B); FIGO, I+II (C); FIGO, III+IV (D); pN0 stage (E); pN1 stage (F).

RNF187 is one of the RING type E3 ligases families containing an N-terminal RING domain that have E3 ubiquitin ligase activity and promote ubiquitylation of substrates,[25] and plays a crucial role in tumoregnesis.[13] The RNF187 protein is considered as a pivotal regulator in controlling cell proliferation, cell cycle and cell signaling by cooperating with or binding to multiple oncogenes such as K-ras and APC etc.[9,13] Recently, previous studies appeared to that RNF187 is acted as an oncogene in human hepatocellular carcinoma and NSCLC due to its capacity to induce epithelial to mesenchymal transitions.[12,26] In recent years, it is uncovered that RNF187 is expressed widespread in human colon carcinoma cells, cervical carcinoma cells and rat pheochromocytoma cell line, and overexpression of RNF187 has been demonstrated to be associated with the proliferation and development of variable human carcinomas, such as human hepatocellular carcinoma, NSCLC, and colon carcinoma. Meanwhile, knockdowning of RNF187 has been shown to inhibit invasion and migration in hepatocellular carcinoma and NSCLC cells.[12,13,26] However, the RNF187 protein expression and its prognostic significance remain unclear in human ovarian carcinoma. In this study, we investigated the expression of RNF187 protein in 147 sample tissues with ovarian carcinoma. Our results uncovered that high level of RNF187 expression was significantly correlated with a malignant phenotype including late FIGO stage, high histologic grade and pN1 stage, respectively. The result indicated that RNF187 might be an oncogene in human carcinomas, and prompt the proliferation and migration of ovarian carcinoma, and inhibit the differentiation of ovarian carcinoma, which was partially agreement with that the previous study reported.[12] Our findings suggested that the key role of RNF187 as an oncogene in the development and progression of ovarian carcinoma. Moreover, these results uncovered that RNF187 functions as an oncogene of malignant phenotype and could be activated in human carcinomas. Meanwhile, univariate and multivariate analysis demonstrated that high expression of RNF187 was closely associated with unfavorable prognosis of patients with ovarian carcinoma, and acted as an independent prognostic factor. These data were consistent with the evidence by other researches,[11,12,26] indicating that activated RNF187 might cause aggressive proliferation of tumors and could be used as a critical biomarker for the evaluation of prognosis in human carcinomas. We conjectured that RNF187 could maintain a certain degree of level in ovarian carcinoma with normal immunity environment, once the status changed aberrantly, RNF187 may be increased

expression by inhibiting silent sequence variants, which could be testified by previous studies reported that RNF187 expression is modulated by NF-$\kappa$B pathway that is closely correlated with tumor immunity.[15,27] Mitogen extracellular signal-regulated kinase/extracellular regulated protein kinases (MEK/ERK) signaling pathway could prompt the formation of RNF187 Lys 63-linkage ubiquitin chains on C-terminal lysine residues that blocking degraded auto-ubiquitylation can stabilize RNF187 protein levels instead of Lys 48 chains and further mediated c-Jun coactivation,[13] suggesting that several underlying molecular mechanism leads to RNF187 upregulation. However, the potential mechanism that RNF187 influences prognosis still unknown and will require further to be investigated. Therefore, we will deeply explore the mechanisms underlying RNF187 linked gene-mediated progression and metastasis of ovarian carcinoma through identifying the receptor, target proteins, and pathways of associated gene in the future experiments.

In a word, this study revealed that examination of RNF187 expression by IHC can be acted as an effective measure in determining the patients with ovarian carcinoma that have a high risk of tumor invasion and metastasis, and elaborates high expression of RNF187 protein as a newly poor independent prognostic factor in ovarian carcinoma, which might be helpful for us to find multiple novel therapeutic targets.

## Author contribution

Xinke Zhang is responsible for the study design. Jiewei Chen and Keming Chen performed the experiments and draft the manuscript. Zhishan Zhou, Lingbo Huang, Yubo Cai, and Hua Tu anticipated in the data analysis and interpretation. All authors read and approved the final manuscript.

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