ORIGINAL ARTICLE

A Correlation Between the Expression of RNF43 Protein and Prognosis in Mucinous Ovarian Carcinoma

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Background: Mucinous ovarian carcinoma (MOC) is an aggressive cancer with poor prognosis. Therefore, the prognostic prediction factor is important. The Ring finger protein 43 (RNF43) may be the new prognostic marker.

Objective: To evaluate the expression of RNF43, and prognostic prediction of RNF43 protein in MOC.

Material and Methods: The study population were patients diagnosed as MOC, between January 2005 and December 2014, selected from Songklanagarind Hospital, Thailand. All cases had paraffine-embed tissue of tumor. Immunostaining for RNF43 (clone ab217787-Abcam, USA) was performed and evaluated expression by one pathologist and one pathology resident. The expression of RNF43, clinical features and survival outcome were evaluated.

Results: There were 104 cases, and all cases showed positive in cytoplasmic staining for RNF43, with high RNF43 expression being found in 86 cases (82.7%). Tumor stage and tumor recurrence were significantly related to RNF43 level of expression (p=0.02 and 0.04, respectively). Approximately 80% of the patients with high RNF43 expression were in the early stage and about 90% showed no tumor recurrence. Patients with high RNF43 expression had longer survival than patients with low RNF43 expression. However, RNF43 expression was not significantly correlated with survival outcome.

Conclusion: MOC showed high expression of RNF43 protein, however, RNF43 expression is not an independent prognostic survival factor by multivariable analysis.

Keywords: Immunohistochemistry; Mucinous ovarian carcinoma; Prognosis; RNF43

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Mucinous ovarian carcinoma (MOC) presents in around 3% of epithelial ovarian cancers, worldwide, and about 20% of all ovarian cancers in Thailand[1,2]. Epithelial ovarian cancer patients, especially those with MOC, usually present in the advanced stage and have a poor survival outcome[3,4]. MOC is usually resistance to chemotherapy, resulting in a high mortality rate[5-8].

A Wingless-related integration site (Wnt) could be a tumorigenesis of mucinous ovarian tumors[9]. An activated Wnt signaling pathway results in epithelial proliferation, and an aberrant Wnt signaling pathway regulates chemoresistance in mucinous ovarian cancer[10-12]. Ring finger protein 43 (RNF43) is an E3 ubiquitin ligase gene situated at chromosome 17q22 that negatively controls the Wnt signaling pathway[13]. A dysregulation of RNF43 results in persistently activation of the Wnt signaling pathway[14]. RNF43 gene mutation has frequently been found in mucinous tumor of ovary and other organs[15-21]. Studies have suggested that Wnt signaling pathway targeted therapy could be an effective treatment of cancer, especially in RNF43 mutated cancer[22-27]. Loss-of function of RNF43 protein expression could predict poor prognosis in patients with gastric cancer, colonic cancer, cholangiocarcinoma, and glioma[28-31]. However, there are few studies regarding the association of RNF43 and MOC as a prognostic factor.

Hence, the objective of the present study was to evaluate the expression RNF43 protein in MOC and evaluate prognostic significance.

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Material and Methods

Study population

The study population were patients diagnosed as MOC between January 2005 and December 2014. They were selected retrospective from the Department of Pathology, Songklanakarin Hospital, Thailand. Clinicopathological information was collected from medical records, including age, side of tumor, tumor size, TMN stage, recurrence of disease, and 5-year survival: the latest follow up time was in 2019. The subjects were MCO cases who underwent primary surgery and completed treatment and clinicopathological reports. Patients who had prior other cancers, received neoadjuvant chemotherapy or inadequate formalin-fixed paraffin-embedded samples were excluded. The result of sample size calculation for testing a difference in the disease hazard with unequal sample was 100 cases. The present study was approved by the Research Ethic Committee, Faculty of Medicine, Prince of Songkla University (REC.61-321-5-1).

Immunohistochemistry

Formalin-fixed, paraffin-embedded pathologic specimens from all histologically confirmed MCO were achieved. Immunohistochemistry (IHC) was performed on formalin-fixed paraffin embedded tissue from selected cases. Anti-RNF43 (clone ab217787-Abcam, USA) primary antibody was stained by using the Leica BOND-MAX automated immunostainner.

Interpretation

All IHC staining cases were reviewed by the pathologist and co-investigator by light microscope. Both cytoplasmic and nuclear staining were evaluated. The intensity of staining was scored as 0 for negative and positive 1+ to 3+. The extensity score of stained cells were recorded as 0 for negative, 1 as positive for less than 10%, 2 as positive for 10% to 50%, and 3 as positive for more than 50% of the cells (Figure 1). The final score combined the score of extensity and intensity with 0 to 1 for negative, 2 to 3 for low expression, and 4 to 6 for high expression[32].

Statistical analysis

The correlation between expression of RNF43 and clinicopathological features of MOC patients were resolved with the chi squared test. Univariable and multivariable survival analyses were performed using the Cox proportional hazards regression model. Survival analysis was determined by Kaplan-Meier method and log-rank test. Significance difference was regulated when the p-value less than 0.05. All statistical analyses were calculated by R program studio 3.3.1 (Rstudio, Boston, USA).

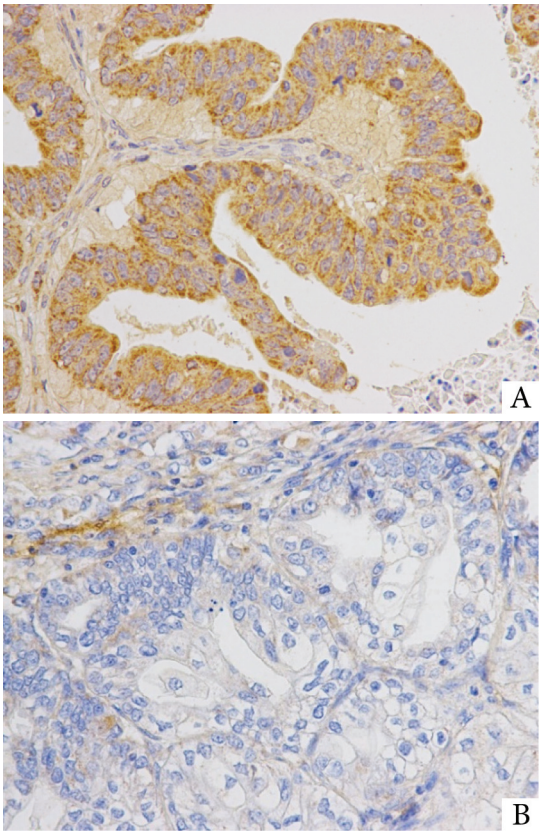

Figure 1. The expression pattern of RNF43 showed positive cytoplasmic staining (intensity=3+) (A). The negative expression reveals no staining of RNF43 protein in the tumor cells (intensity=0) (B).

Results

One hundred and four cases of MOC were included in the present study. All cases showed RNF43 protein expression in cytoplasm. The results showed that 86 cases (82.7%) had high expression of RNF43 protein. RNF43 protein expression was associated with tumor stage and tumor recurrence (p=0.02 and 0.04, respectively). In the low expression of RNF43 group, eight out of 18 cases (44.4%) were in the advanced stage. Early-stage mucinous ovarian cancer was found in 67 out of 86 cases (77.9%) with high expression of RNF43. In the low RNF43 protein expression group, 13 out of 18 cases (72.2%) had recurrence of the disease. In the high RNF43 expression group, 78 out of 86 cases (90.6%) did not have recurrence of the disease. Other factors, such

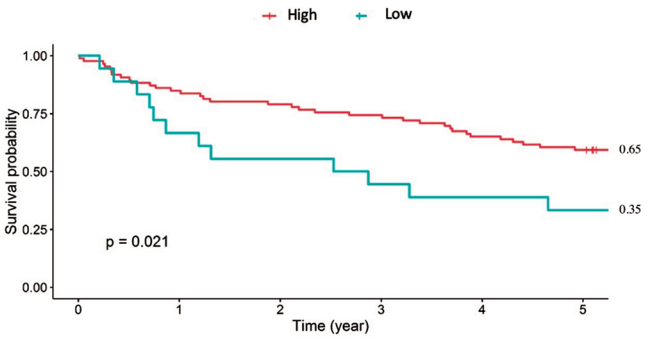

Figure 2. Kaplan-Meier analysis of mucinous ovarian carcinoma patients with low expression of RNF43 protein demonstrated a longer survival than patients with high expression of RNF43 protein (p=0.02).

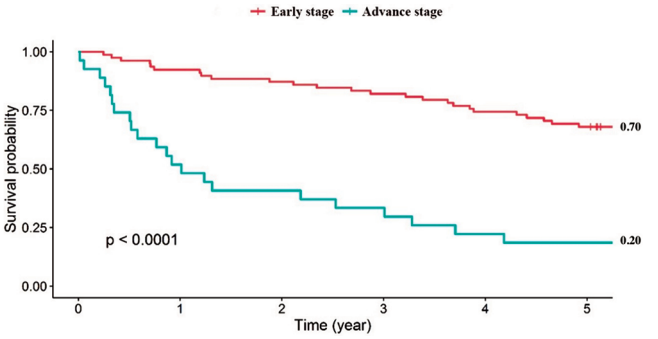

Figure 3. Kaplan-Meier analysis comparing between patients in early stages (stage 1 and 2) and advanced stages (stage 3 and 4) revealed a longer survival in early-stage patients (p<0.001).

Table 1. Clinicopathological characteristics and RNF43 protein expression in mucinous ovarian carcinoma

Characteristic	RNF43 protein expression		p-value
	High expression (n=86)	Low expression (n=18)	
Age; mean [SD]	48.6 [13.5]	46.3 [13.4]	0.50
Parity; n (%)			0.46
Null	24(29.3)	3 (17.6)	
Para	58(70.7)	14 (82.4)	
TNM stage; n (%)			0.02
Stage 1	53 (61.6)	5 (27.8)	
Stage 2	14 (16.3)	5 (27.8)	
Stage 3	15 (17.4)	8 (44.4)	
Stage 4	4 (4.7)	0 (0.0)	
Laterality; n (%)			0.08
Right	39 (46.4)	6 (33.3)	
Left	35 (41.7)	6 (33.3)	
Both sides	10 (11.9)	6 (33.3)	
Size; median (IQR)	14 (12.0,17.0)	12 (8.2,18.5)	0.36
Recurrent; n (%)			0.04
Yes	8 (9.3)	13 (72.2)	
No	78 (90.7)	5 (27.8)	

SD=standard deviation; IQR=interquartile range

as age, tumor site, tumor size, and parity showed no statistical significance related to RNF43 expression (Table 1).

Kaplan-Meier survival curve demonstrated that five years overall survival (OS) of patients with high expression of RNF43 had significantly longer than RNF43 low expression patients (0.65 and 0.35, p=0.02) (Figure 2). The mean survival time in years, of patients with high expression of RNF43 and low expression were 4.0 and 2.8, respectively. Regarding tumor stages, patients with early stages showed longer OS than patients in an advanced stages, with statistical significance (0.70 and 0.20, p<0.001) (Figure 3). However, multivariable analysis indicated that RNF43 protein expression was not an independent prognostic factor for patient survival (Table 2).

Discussion

In the present study, most patients with MOC showed high cytoplasmic RNF43 protein expression. This result coincides with the studies of RNF43 altering in MCO, from the Exome sequencing method; in which 21.0% of cases exhibited RNF43

Table 2. Univariable and multivariable analysis of different prognostic factor in RNF43 protein expression and mucinous ovarian carcinoma specimens

Characteristics	Univariable analysis hazard ratio (95% CI)	p-value	Multivariable analysis hazard ratio (95% CI)	p-value
RNF43 expression	2.14 (1.11 to 4.13)	0.02	1.32 (0.64 to 2.73)	0.46
Age	0.99 (0.98 to 1.02)	0.66	1.00 (0.98 to 1.00)	0.87
TNM stage		<0.001		<0.001
Stage 1	1.00		1.00	
Stage 2	1.79 (0.77 to 4.15)		1.48 (0.59 to 3.71)	
Stage 3	5.90 (3.03 to 11.49)		4.98 (2.46 to 10.08)	
Stage 4	5.69 (1.66 to 19.51)		5.20 (1.47 to 18.38)	
Tumor size	0.97 (0.92 to 1.03)	0.93	0.99 (0.94 to 10.46)	0.73
Recurrence	2.23 (1.10 to 4.50)	0.04	1.49 (0.66 to 3.35)	0.35

CI=confidence interval

mutations[15,16]. Loss-of-function mutation of RNF43 relates to constantly stimulating the Wnt signaling pathway that causes cell proliferation and tumorigenesis. The present study showed that there is RNF43 protein altering in MOC.

In the present study, most of patients were in the early stages of cancer because of guideline of treatment that mainly used tumor removal in the early stages and used neoadjuvant or palliative chemoradiation in the advance stages. Patients with high RNF43 protein expression were in the early stage more than in an advanced stage. Most patients with high RNF43 expression showed no tumor recurrence. Therefore, RNF43 protein expression in tumor cells might be associated with tumor progression as well as tumor staging. High expression of RNF43 in tumor cells might inhibit the tumor cell growth, and therefore a protective factor for tumor progression.

However, RNF43 protein expression was not an independent prognostic factor in patients with MOC. This result is unlikely related to other cancers, such as cholangiocarcinoma, gliomas, and gastric cancer, which were associated with poor survival and served as an independent prognostic factor. Cox regression analysis demonstrated that tumor stage was only an independent prognostic factor, whereas, RNF43 was not an independent prognostic factor in patients with MOC.

Conclusion

Mucinous ovarian cancer showed high expression of RNF43 protein. Tumor stage and tumor recurrence were associated with the expression of RNF43 protein. However, RNF43 expression was not an independent prognostic survival factor by multivariable analysis.

What is already known about this topic?

The RNF43 controlled cell proliferation and the growth of tumor cells. Loss expression of RNF43 in many cancers indicated the poor prognosis. However, there was limited study of correlation between expression of RNF43 and prognosis of mucinous ovarian cancer.

What does this study add?

The results of this study indicated that most of mucinous ovarian cancer cells expressed RNF43 protein in various level by immunohistochemical staining. Patients with high RNF43 protein expression were in the early stage and showed no tumor recurrence. However, RNF43 protein expression was not associated with prognostic outcome.

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Conflicts of interest

No conflict of interest declared.

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