ORIGINAL RESEARCH

Analysis of Ki67 Protein Expression and Clinicopathological Features in Patients with Peritoneal Mesothelioma

Dujuan Yang, BM; Yeping Zhou, BM; Kongdan Lv, MM

ABSTRACT

Background • At present, there are many treatments for peritoneal mesothelioma, but the treatment of peritoneal mesothelioma is still facing great challenges. Distant metastasis is the main cause of poor prognosis and death of patients with peritoneal mesothelioma. Ki67 is a cell proliferation marker. In recent years, it has been found to be used as a molecular marker for the diagnosis, treatment and prognosis of different tumor cells. Ki67 has been shown to play a crucial role in the occurrence and development of a variety of cancers. However, the clinical significance and biological function of Ki67 in peritoneal mesothelioma remain poorly understood.

Purpose • To clarify the expression of Ki67 in peritoneal mesothelioma (PC), and to explore the relationship between the expression level of Ki67 and the clinicopathological parameters and prognosis of patients with PC, and to explore the potential of Ki67 as a therapeutic target and prognostic biomarker for PC.

Methods • TIMER database was used to compare the expression levels of Ki67 mRNA and protein in mesothelioma tissues and adjacent tissues. The relationship between the expression level of Ki67 in mesothelioma and clinicopathological characteristics, and the relationship between the expression level of Ki67 and the level of immune infiltration in mesothelioma were analyzed. The prognostic value of Ki67 in mesothelioma patients was predicted, and the overall survival curve was drawn according to the follow-up data. LinkedOmics database and GSEA were used to perform co-expression analysis and enrichment analysis of Ki67, respectively.

Results • Bioinformatics analysis showed that Ki67 was highly expressed in peritoneal mesothelioma ($P < .01$). Immunohistochemistry showed that the positive rate of Ki67 in peritoneal mesothelioma was high, and the number of Ki67 positive cases was 62.0% (31/50 cases). Univariate analysis showed that TNM stage ($P = .007$), asbestos ($P < .001$), chemotherapy ($P < .001$), and Ki67 expression level ($P = .029$) were associated with prognosis. Multivariate analysis showed that Ki67 expression level ($P = .039$) and TNM stage ($P = .029$) were independent risk factors for the prognosis of peritoneal mesothelioma. Peritoneal mesothelioma patients with high Ki67 expression have poor OS. In addition, Ki67 is also associated with the immune infiltration of mesothelioma.

Conclusion • Ki67 is highly expressed in peritoneal mesothelioma. Ki67 protein plays an important role in the development of peritoneal mesothelioma and is one of the important factors to evaluate the prognosis of patients with peritoneal mesothelioma. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Membranous mesothelioma is a rare tumor originating from the peritoneal mesothelium and Epithelium. Miller and Wynn first described the disease in 1908.[1] The early symptoms of the disease are mostly hidden and nonspecific, with abdominal distention and abdominal pain caused by ascites as the main symptoms, followed by emaciation, fatigue, poor appetite, nausea, vomiting, low heat, night sweat, abdominal mass, abnormal urine, and stool.[2] The diagnosis mostly relies on peritoneal biopsy, Previous reports have found that about 40% -60% of patients have metastasis at diagnosis, and the median survival time of untreated patients is less than 1 year.[3-5] According to statistics, about one person in a million people is diagnosed with malignant peritoneal mesothelioma every year, and about 800 people in the United States are diagnosed with this disease every year, of which the incidence rate of men and women is similar. Previous studies have shown that the number of new cases and deaths of MPM

Table 1. Baseline characteristics of patients and distribution of Ki67 mutations [n (%)]

Items	Total number of people (n = 50)	Ki67		*P* value
		Positive (n = 31)	Negative (n = 19)	
Age (years)				.879
≤ 50	23(46.0)	14(45.16)	9(47.37)	
> 50	27(54.0)	17(54.84)	10(52.63)	
Gender				.285
Male	31(62.0)	21(67.74)	10(52.63)	
Female	19(38.0)	10(32.26)	9(47.37)	
Asbestos exposure				.019
Yes	42(88.0)	28(90.32)	11(57.89)	
No	6(12.0)	3(9.68)	8(42.11)	
Ascites				.893
Exist	43(86.0)	27(87.10)	16(84.21)	
Null	7(14.0)	4(12.90)	3(15.79)	
Pleural plaques				.944
Exist	26(52.0)	16(51.61)	10(52.63)	
Null	24(48.0)	15(48.39)	9(47.37)	
Lymph node metastasis				.464
Positive	31(62.0)	18(58.06)	13(68.42)	
Negative	19(38.0)	13(41.94)	6(31.58)	
TNM stage				.301
I+II	13(36.0)	6(19.35)	7(63.15)	
III+IV	37(64.0)	25(80.65)	12(36.84)	
Chemotherapy				.659
Exist	3(6.0)	2(6.45)	1(5.26)	
Null	47(94.0)	29(93.55)	18(94.4)	

every year is increasing year by year. The incidence of MPM is mostly seen in the elderly, with a median age of 64 years.[4-7] The etiology is often related to asbestos exposure, with occupational exposure as the main form. The average incubation period is 41.9 years for males and 36.8 years for females, with a linear trend increasing.[8]

Ki67 is a nuclear protein composed of two polypeptide chains with relative molecular weight of 345kD and 395kD. Ki67 (MKi67) gene is located on chromosome 10q25-ter and consists of 15 exons and 14 introns. Studies have shown that Ki67 is a nuclear protein associated with and possibly required for cell proliferation; therefore, the nuclear expression of Ki67 can be detected by immunohistochemistry (IHC) to assess tumor proliferation.[9,10] Studies have shown that its function is linked to chromatin and closely related to cell mitosis, so it is one of the most widely used markers of proliferating cells.[11,12] The expression level of Ki67 protein can well reflect the proliferative activity of tumor cells. It is only expressed in proliferating cells and low expression in normal tissues. High expression of Ki67 is an important marker of active cell proliferation, which is not limited to non-small cell lung cancer, but is related to the occurrence, development, metastasis and prognosis of a variety of tumors such as breast cancer, colon cancer and bladder cancer.[13,14] Ki67 is a nuclear protein closely related to cell proliferation and may be necessary for cell proliferation.[15-17] Ki67 is significantly increased in the active phase of cell division, which is considered to be an important indicator reflecting the proliferation activity of tumor cells. At present, it is widely used as a proliferation marker of most tumor cells in clinical practice. Similarly, in mesothelioma, Ki67 has become a molecular biological indicator in the routine clinical examination of mesothelioma patients.[18,19]

In this paper, the expression and prognostic significance of Ki67 in mesothelioma tissues were analyzed by bioinformatics and immunohistochemistry. Firstly, we analyzed the expression of Ki67 in mesothelioma using TIMER, Kaplan-Meier plotter, CCLE and UALCAN databases. Secondly, we used UANCAL database to analyze the relationship between the expression level of Ki67 and clinicopathological features. The Kaplan-Meier plotter database was then used to predict the prognostic value of Ki67 in patients with mesothelioma, and was validated and analyzed on clinical specimens. In addition, the relationship between Ki67 expression and the level of mesothelioma immune invasion was explored by using TIMER database. Finally, the co-expression and enrichment of Ki67 were analyzed using the LinkedOmics database and GSEA respectively. In this paper, we will clarify the expression of Ki67 in peritoneal mesothelioma, analyze the relationship between the expression level of Ki67 and the prognosis of patients with peritoneal mesothelioma, and evaluate the possibility of Ki67 as a biomarker for the prognosis of peritoneal mesothelioma.

MATERIALS AND METHODS

General Information

This study is a retrospective study. Paraffin specimens of 29 patients with peritoneal mesothelioma obtained by B-ultrasound guided puncture biopsy or operation in our hospital from September 2015 to September 2021 were collected, and paraffin specimens of 21 patients with peritoneal mesothelioma were collected during the same period. These patients were newly diagnosed with mesothelioma and had not received any antitumor therapy before surgery. All the above patients have signed written informed consent and confidentiality agreement, and have been approved by relevant ethics committees. Details are shown in Table 1.

Inclusion criteria: (1) histologically or cytologically confirmed diagnosis of peritoneal mesothelioma; (2) complete and detailed electronic medical records with records of regular review and follow-up results.

Exclusion criteria: (1) non-peritoneal mesothelioma or unclear diagnosis; (2) severe missing information or no pathological results; (3) no follow-up data at the beginning of the study and those who had been lost to follow-up. Information was collected from 4 dimensions: (1) demographic characteristics, disease history, family genetic history, and preoperative personal history; (2) clinical information such as imaging and preoperative serological tests; (3) treatment modalities; (4) study outcomes. Information including recurrence, metastasis, death, survival, refusal, and loss was obtained through outpatient medical records, readmission records, and telephone follow-ups.

All patients were followed up until May 1, 2023. Regular outpatient follow-up was used to follow up the corresponding time points of disease recurrence, metastasis and death as the follow-up end point, and the overall survival (OS) of nearly 20 months was observed.

Immunohistochemical detection and judgment standard

Mouse anti-human Ki67 monoclonal antibody (clone number: MIB-1, dilution ratio: 1:50) was purchased from

China Fir Biotechnology Co., LTD., Beijing, China, operated strictly according to the instructions. TBS buffer: 1000 mL solution containing 6.06 g Tris, 0.88g NaCl, 0.5 mL Tween20 adjusted to pH 7.5±0.2 aqueous solution; DAB staining solution: DAB staining solution (produced by Gene Technology (Shanghai) Co., LTD., Humin, No. 20140019). All the peritoneal tissue samples were stained by immunohistochemistry under the same condition. The slides were fixed with 10% neutral formaldehyde, routinely embedded in paraffin and sectioned with a thickness of 4μm, routinely deparaffinized with xylene, and dehydrated with gradient alcohol. After deparaffinized and hydrated, the slides were rinsed with TBS buffer for 3 minutes, 3 ×, the primary antibody was added, and after incubation overnight, the biotin-labeled secondary antibody was dropped, and the color was detected by DAB (100μL per slide). Contrast counterstained with hematoxylin, and the slides were routinely dehydrated, transparent, dried and sealed with neutral gum. Most Ki67-positive cells showed nuclear staining and brownish yellow, and a few showed weak cytoplasmic staining.

Bioinformatics prediction methods

TIMER(https://cistrome.shinyapps.io/timer/) is an online database for the comprehensive study of molecular signatures of tumor-immune interactions. In this thesis, using TIMER database, we investigated the differential expression of Ki67 in all TCGA tumors compared with normal tissues.

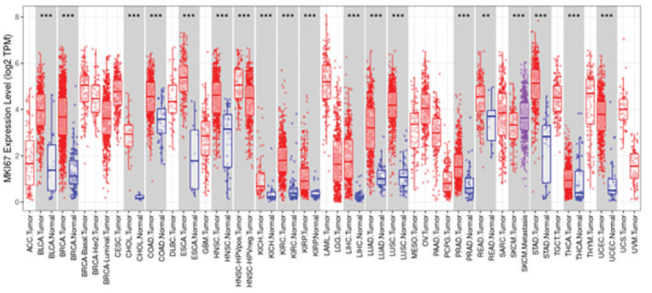
UALCAN (http://ualcan.path.uab.edu) is an open database that allows both the study of gene expression levels and the comparison of primary tumors with clinicopathological features. In this thesis, we first selected the term "CPTAC" in the first screen to obtain the protein expression of Ki67 in peritoneal mesothelioma. Secondly, we selected the "TCGA" term in the UALCAN database and entered the following screening criteria: the gene name "Ki67" was entered, and "peritoneal mesothelioma" was selected in the "TCGAdataset" to obtain the relationship between gene expression and relevant clinicopathological features. In this paper, we also used the "survival" function of this database for prognostic analysis.

CCLE (http://portals.broadinstitute.org/ccle) is used to determine the new cancer markers driven correlation, provides resources for acceleration of cancer research. In this paper, we analyzed the mRNA levels of Ki67 in multiple cancer cell lines through the CCLE database.

In this paper, we take advantage of the features of survival analysis in GEPIA(http://gepia.cancer-pku.cn/). After entering the GEPIA database interface, enter the gene Ki67 and click "survival analysis", select "peritoneal mesothelioma" in the box of "data sets selection (cancer name)", and the other options are the default Settings. The relationship between Ki67 expression in peritoneal mesothelioma and overall survival can be obtained.

Kaplan - Meierplotter (http://kmplot.com/analysis/) is mainly used for predicting the prognosis of online database.

Figure 1. Expression levels of Ki67 in pan cancer



In this paper, we investigated differential gene expression analysis in tumor, normal and metastatic tissues and the association of Ki67 expression with peritoneal mesothelioma survival using the Kaplan-Meierplotter database.

LinkedOmics (http://www.linkedomics.org/login.php) it covers TCGA project of the 32 types of cancer and more than a total of 11158 patients of omics data and clinical data. In this paper, we obtained the co-expressed genes of Ki67 through the LinkedOmics database and performed GO and KEGG analyses. Gene Ontology biological process (GO_BP), Gene Ontology cellular component (GO_CC), Gene Ontology molecular function (GO_MF) and KEGG pathways were analyzed by GSEA in LinkFinder module. The rank criterion was FDR<0.05, and 500 simulations were performed.

Statistical analysis

We used Logistic regression to analyze the relationship between Ki67 expression and clinicopathological features. Univariate and multivariate Cox regression methods were used to compare the prognostic value of Ki67 expression as a determining factor. In the statistical description of this study, the measurement data were described by mean ± standard deviation (±s) if they were normal distribution, or by median and quartile if they were not. Count data were described by frequency and percentage (number of cases, %). $P < .05$ was considered statistically significant. All statistical analyses were analyzed using SPSS version 23.0 (IBMCorporation, Armonk, NY, USA) or GraphPad Prism 5.0 (Graphpad Software, LaJolla, CA, USA).

RESULTS

TIMER database analysis of Ki67 expression levels in pan-cancer

We used the TIMER database to analyze the differences in Ki67 expression in all TCGA-stowed tumors. The results showed that Ki67 expression was significantly higher in uroepithelial carcinoma of the bladder, invasive carcinoma of the breast, colon cancer, esophageal cancer, liver cancer, mesothelioma, renal suspensory cell carcinoma, and head and neck cancer than in normal tissues (Figure 1).

CCLE database analysis of Ki67 expression levels in peritoneal mesothelioma and systemic tumor cell lines

To further validate the expression of Ki67 in different human tumors, we analyzed the mRNA sequence data at the

Figure 2. Expression levels of Ki67 in different tumour cell lines

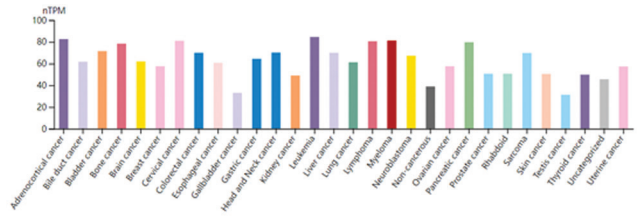


Figure 3. Kaplan-Meier plotter analysis of Ki67 expression levels in mesothelioma tissue, normal tissue and metastatic tissue

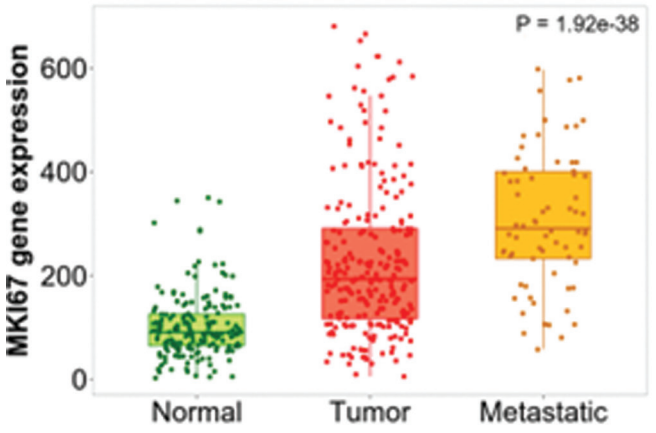


Figure 4. UALCAN database analysis of the relationship between Ki67 gene expression levels and the clinicopathological and molecular characteristics of mesothelioma; (A) Ki67 (B) Lymph node status (C) Gender (D) Molecular typing (E) Age (F) Tumour stage

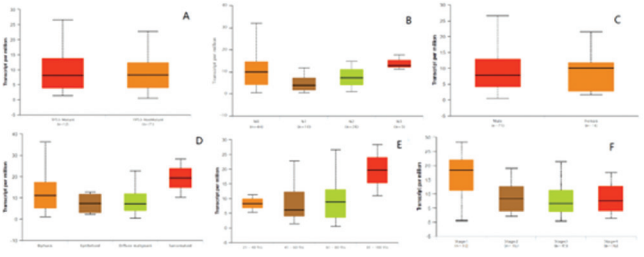
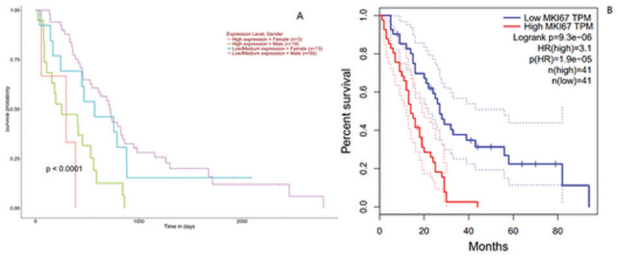


Figure 5. Survival curve of Ki67 gene; (A) Overall survival curve (OS) of Ki67 in Kaplan Meier plot; (B) OS of Ki67 in GEPIA



cellular level using the CCLE database. As shown in the figure, Ki67 was highly expressed in tumors such as breast, oesophageal, pancreatic, and colorectal cancers, while it was lowly expressed in gallbladder, kidney and testicular cancers (Figure 2). These data suggest that Ki67 has different expression levels in different tumors, implying that Ki67 may perform multiple functions in various tumors.

Kaplan-Meier plotter database analysis of Ki67 expression levels

We used the Kaplan-Meier plotter database to analyze the mRNA expression of Ki67 in mesothelioma tissue, normal tissue, and metastatic tissue. The results showed that Ki67 expression was significantly elevated in mesothelioma ($P < .001$) (Figure 3).

UALCAN database analysis of Ki67 expression in relation to clinicopathological features

To investigate whether Ki67 expression levels are associated with the development of mesothelioma, we analyzed the correlation between Ki67 expression and mesothelioma clinicopathological features using the UALCAN database. For the Ki67 mutation, Ki67 expression in the mutant phenotype was not significantly different from the wild type ($P > .05$) (Figure 4A). According to the lymph node metastasis status, Ki67 expression was lower in NO, N1, and N2 mesothelioma tissues than in N3 ($P < .001$), with no significant difference in Ki67 expression in NO, N1, and N2 mesothelioma tissues ($P > .05$) (Figure 4B). As shown in Figure 4, in terms of gender, Ki67 expression in male mesothelioma tissues was not significantly different from that in female mesothelioma tissues ($P > .05$) (Figure 4C). Among the various subtypes of mesothelioma, we could find significant differences in Ki67 expression in sarcomatoid mesothelioma compared to diffuse malignant mesothelioma, epithelioid mesothelioma, and biphasic mesothelioma ($P < .01$) (Figure 4D). For age, Ki67 expression was significantly higher in mesothelioma tissues aged 21-40, 41-60, and 61-80 years than in those aged 81-100 years. There was no significant difference in Ki67 expression in mesothelioma tissues between 21-40, 41-60, and 61-80 years of age ($P > .05$) (Figure 4E). Finally, according to the stage of the disease, a comparison between stages I-IV revealed that Ki67 expression was lower in stage I-III mesothelioma tissues than in stage IV mesothelioma tissues ($P < .01$) (Figure 4F).

High expression of Ki67 is associated with poor prognosis in patients with mesothelioma

We first used Kaplan Meier Plotter database to analyze the relationship between Ki67 expression and OS in mesothelioma patients. The results showed that Ki67 expression level was high, OS in mesothelioma patients was short ($P < .001$), and OS in patients was not correlated with gender ($P > .05$) (Figure 5A). In addition, we also used the GEPIA database to verify the relationship between Ki67 expression and OS in mesothelioma patients, which is similar

to the results of the KMP database. The GEPIA database also showed that the highly expressed Ki67 was associated with shorter OS in mesothelioma patients ($P < .01$) (Figure 5B).

Univariate analysis showed that TNM stage ($P = .007$), asbestos ($P < .001$), chemotherapy ($P < .001$), and Ki67 expression level ($P = .029$) were associated with OS. Then, we included the four indicators that were significant in univariate analysis into the COX proportional hazards model for multivariate analysis. The results showed that only Ki67 expression level ($P = .039$) and TNM stage ($P = .029$) were independent prognostic factors (Table 2). The severity of the disease is related to the stage of TNM. The higher the stage, the more severe the disease, the more difficult to treat and the worse the prognosis. Similarly, the expression level of Ki67 is also related to the stage of the tumor.

TIMER database analysis of Ki67 expression correlates with markers of different immune cell subpopulations

We explored the correlation between Ki67 expression and the level of immune infiltration in mesothelioma through the TIMER database. The results showed that Ki67 expression was significantly positively correlated with B cells ($P < .001$, r=0.403), CD4$^+$T cells ($P < .05$, r=0.228), CD8$^+$T cells (P<0.001, r=0.085), macrophages (P<0.001, r=0.145), and dendritic cells (P<0.001, r=0.445) correlation (Figure 6).

LinkedOmics database analysis of Ki67 gene co-expression analysis

We assessed the co-expression pattern of Ki67 in the mesothelioma cohort using the LinkedOmics functional module. The results showed that 1569 of these genes (shown by red dots) were significantly positively correlated with Ki67 expression and 4973 genes (shown by green dots) were significantly negatively correlated with Ki67 expression (Figure 7A). The 50 genes most associated with Ki67 expression are shown separately in the heat map, with Figure 7B showing the positively associated genes and Figure 7C showing the negatively associated genes. According to the LinkedOmics database analysis, KIF11 (kinesinfamilymember11) was the most associated gene with Ki67 in mesothelioma.

LinkedOmics database analysis of Ki67-related co-expressed genes enrichment analysis

Based on the results of Ki67 co-expression gene analysis, GSEA's GO terminology analysis showed that genes associated with Ki67 are mainly located in the lateral side of the cell membrane, mitochondria, replication forks, cytoplasmic fraction, and tertiary granules, they are mainly involved in defense responses against other organisms, granulocyte activation, and cellular material exchange functions, and they likewise act as structural in β-binding protein and transcriptional repressor activation components (Figure 8A-C). As analyzed by the KEGG pathway, they are enriched in Hedgehog signaling, allograft rejection, and carbon metabolism (Figure 8D).

Figure 6. Relationship between Ki67 expression and the level of mesothelioma immune infiltration

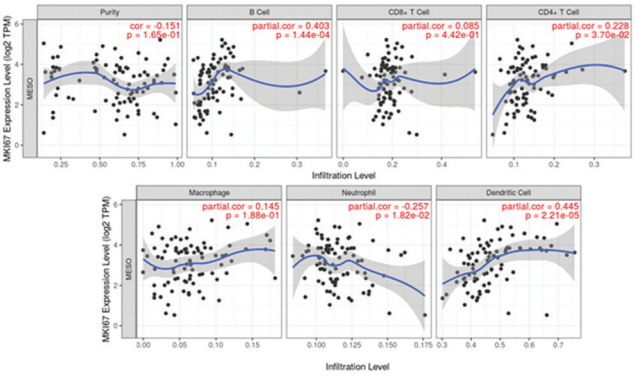


Figure 7. Co-expressed genes of Ki67 in mesothelioma

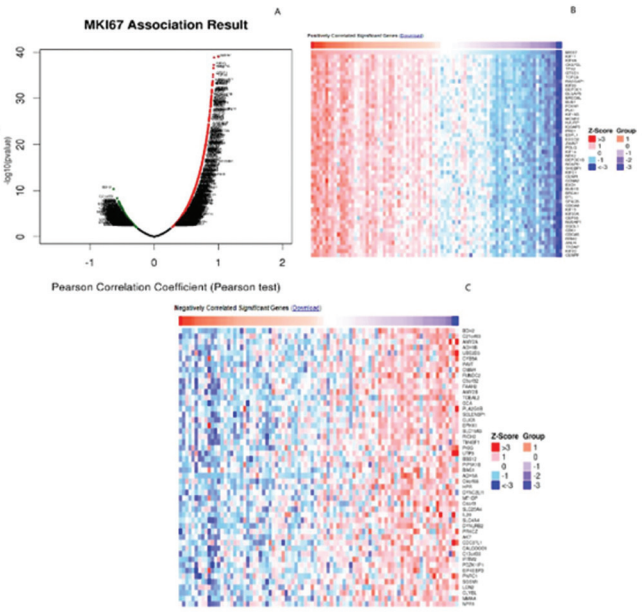


Figure 8. Enrichment analysis of Ki67 co-expressed genes using LinkedOmics database

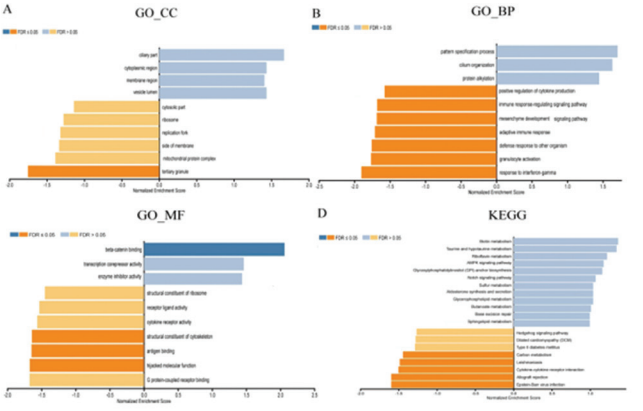


Figure 9. Immunohistochemical expression of Ki67 in peritoneal Mesothelioma; (A,B) Peritoneal Mesothelioma (C,D) pericancerous tissue of peritoneal Mesothelioma

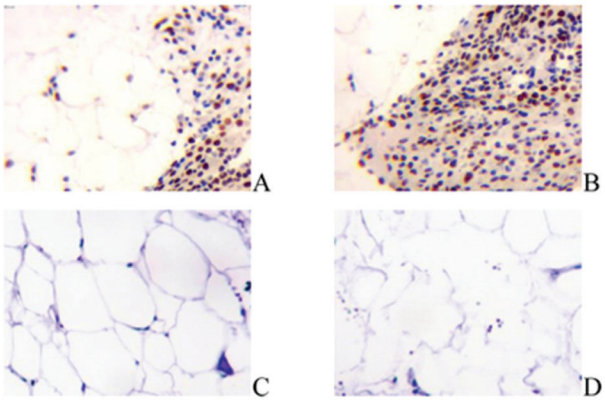


Figure 10. OS curve plotted based on Ki67 protein expression

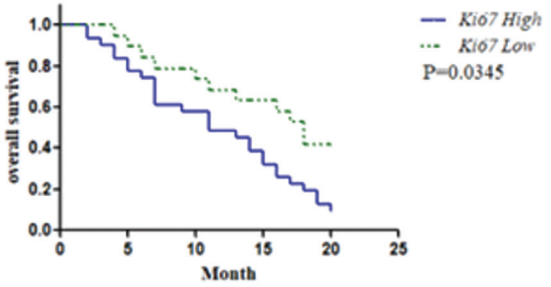


Table 2. Univariate and multivariate analysis of clinicopathological factors affecting survival in mesothelioma patients

Clinical indicators	Single factor hazard ratio (95%CI)	*P* value	Multi factor hazard ratio(95%CI)	*P* value
Gender	1.673 (1.152-2.429)	.17	-	-
Age	2.145 (1.497-3.073)	.34	-	-
Asbestos	4.327 (2.508-7.465)	<.001	2.618 (1.021-6.712)	.065
TNM staging	2.519 (1.787-3.549)	<.001	1.856 (0.889-3.875)	.029
Ascites	1.312 (0.931-1.849)	.12	-	-
Pleural plaques	1.42 (0.983-2.052)	.062	-	-
Chemotherapy	0.88 (0.593-1.306)	.036	2.423 (1.268-4.629)	.067
Ki67 expression level	1.438 (1.039-1.991)	.029	1.743 (1.029-2.953)	.039

Ki67 Expression in Peritoneal Mesothelioma and Adjacent to Carcinoma

We used immunohistochemical experimental methods to verify the conclusions about Ki67 expression in the above database. We got the results that Ki67 staining showed nuclear expression in peritoneal Mesothelioma tissue, and Ki67 positive expression rate was 62.0% (31/50 cases) in peritoneal Mesothelioma tissue, and 38.0% (19/50 cases) in precancerous tissues of peritoneal Mesothelioma. There was a statistically significant difference between the two expressions ($P < .05$), Figure 9.

The relationship between Ki67 expression and clinical pathological features

We verified the conclusion on Ki67 expression in the above database by immunohistochemical experiments. The results showed that Ki67 staining showed nuclear expression in peritoneal mesothelioma, and the positive rate of Ki67 in peritoneal mesothelioma was 62.0% (31/50 cases), and the negative rate was 38.0% (19/50 cases). The expression difference between the two groups was statistically significant ($P < .05$). We further analyzed the relationship between Ki67 expression in peritoneal mesothelioma tissues and age, asbestos exposure, lymph node status, pleural plaque, ascites, tumor size and TNM stage of the patients. Chi-square test was used to analyze the results as shown in the following table. A total of 50 patients were included in this study, of whom 27 (54.0%) were older than 50 years; 31 males (62.0%); chemotherapy 47 (94.0%); Lymph node metastasis was present in 31 cases (62.0%); TNM stage III+IV in 37 cases (74.0%); 31 cases were positive for Ki67; and there were 41 cases (82.0%) of asbestos exposure. The expression of Ki67 protein in mesothelioma tissues was not correlated with age, sex, lymph node metastasis or chemotherapy, and the difference was not statistically significant ($P > .05$). The positive expression rate of Ki67 protein in patients with asbestos exposure was 90.32%, while that in patients without asbestos exposure was 57.89%, the difference was statistically significant ($P = .019$). (Table 2).

Relationship between Ki67 expression and prognosis based on immunohistochemical analysis

We followed up with 50 patients with peritoneal mesothelioma mentioned above to verify the relationship between Ki67 expression and survival and prognosis of mesothelioma patients. The endpoint of follow-up was May 2023. According to the clinical follow-up data and Ki67 expression, Kaplan Meier method was used to draw the total survival curve, and the impact of Ki67 protein high expression and low expression on the total survival time was compared. As shown in the figure below, the total survival time of the Ki67 high expression group was shorter than that of the Ki67 low expression group in peritoneal mesothelioma patients ($P = .0345$), (Figure 10).

DISCUSSION

Peritoneal mesothelioma is a rare disease of the peritoneum, which lacks clinical specificity. The diagnosis depends on the peritoneal biopsy pathology. Immunohistochemical staining is of great significance for the diagnosis. Peritoneal mesothelioma is highly aggressive, and its incidence and mortality are still increasing worldwide. The incidence of peritoneal mesothelioma is mainly related to asbestos exposure, and the prognosis of patients is poor, with a 5-year survival rate of only 5%. At present, the diagnostic standard of peritoneal mesothelioma is mainly laparoscopic biopsy, which lacks accurate early diagnostic markers. Therefore, the discovery of new tumor markers can effectively improve the detection rate of early peritoneal mesothelioma, so as to provide timely treatment for patients. The research on its mechanism and prognosis has become the focus of attention. Different scholars have different

conclusions on the prognostic factors of peritoneal mesothelioma. Yan et al.[20] reported that tissue typing, absence of lymph node metastasis, surgery, and intraperitoneal hyperthermic perfusion chemotherapy (HIPEC) are independent prognostic factors. DAMHUIS et al.[21] reported that age and gender are independent prognostic factors. The occurrence, development, and metastasis of tumors are complex processes involving multiple steps, factors, stages, and genetic changes, involving multiple factors. Ki67 antigen is currently a recognized nuclear proliferation marker and one of the most reliable indicators for detecting tumor cell proliferation activity, which is related to the prognosis of various tumors.[22] There are few reports about Ki67 expression in peritoneal mesothelioma, which is mainly related to the relatively rare peritoneal mesothelioma. Ki-67 antibody is associated with various types of malignant lesions. It is a cell proliferation antigen that is present in the nucleus and can be expressed in all cell cycle stages except G0 phase. The expression level of Ki-67 reflects the proliferative activity of cells, and its expression is enhanced in precancerous lesions and tumors. Ki67 is a cell proliferation marker that detects the proportion of cells in the cell cycle. The higher the Ki-67 positive rate, the greater the proportion of tumor cells in the growth cycle, the faster the growth rate of the tumor, and the monitoring of the therapeutic effect becomes more and more important. Ki67 can be used as a reliable indicator to evaluate the proliferative activity of tumor cells after treatment. By continuously monitoring the changes of Ki67 during treatment, the treatment plan can be adjusted in time and the therapeutic effect can be improved. For the first time, we analyzed the relationship between Ki67 and peritoneal mesothelioma, explored the relationship between the expression level of Ki67 and the clinicopathological parameters and prognosis of patients with peritoneal mesothelioma, and explored whether Ki67 has the potential to be a therapeutic target and a biomarker for evaluating the prognosis of peritoneal mesothelioma.

In this study, 50 patients with peritoneal mesothelioma were enrolled. The clinicopathological characteristics and related prognostic immunohistochemical indicators of the tumor were analyzed in detail. It was found that the clinicopathological prognostic indicators affecting the survival of patients included asbestos exposure, Ki-67 expression, TNM staging, etc. Multivariate analysis showed that asbestos exposure history, TNM stage and Ki-67 expression were independent prognostic factors. Ki67 is a marker of cell proliferation in all phases of the cell cycle, represents the antigen most frequently used to assess the proliferative activity of cancer, and is also a clear prognostic marker for a variety of tumors. Belderbos[10] et al. reported that patients with high Ki67 expression had a poor prognosis in the treatment of malignant pleural mesothelioma by extended pleurectomy and decortication. All patients with high Ki67 expression (>10%) died within 30 months, while those with low Ki67 expression had a median OS of 44.5 months. Asbestos exposure is the most common cause of pleural mesothelioma, and about 33 to 50% of patients with diffuse malignant peritoneal mesothelioma reported in the literature have a history of asbestos exposure.

This study explored for the first time the role of Ki67 in peritoneal mesothelioma and confirmed that Ki67 is highly expressed in mesothelioma and predicted that high Ki67 expression may be closely associated with poor prognosis in patients with peritoneal mesothelioma. To obtain a reliable result, we explored Ki67 expression using several online databases. The results showed that both mRNA and protein Ki67 were highly expressed in peritoneal mesothelioma. The high expression of Ki67 was significantly correlated with lymph node metastasis, Ki67 mutation, tumor stage, subtype and age of patients with peritoneal mesothelioma ($P < .05$). In an immunohistochemistry-based analysis, the results showed that Ki67 expression was significantly correlated with the presence of asbestos exposure ($P = .019$). Subsequently, we explored the prognostic potential of Ki67 in mesothelioma using the Kaplan-Meier and GEPIA databases, both of which suggested that mesothelioma patients with high Ki67 expression had a shorter OS, and finally also confirmed these results on immunohistochemistry-based analysis. In univariate and multivariate analysis of clinicopathological factors affecting the survival of patients with peritoneal mesothelioma, Ki67 expression level ($P = .039$) and TNM stage ($P = .029$) were found to be independent prognostic factors. These findings together clarify Ki67 expression as a potential biomarker for predicting the prognosis of peritoneal mesothelioma.

In addition, the relationship between the expression of Ki67 and the degree of immune infiltration in mesothelioma was also an important aspect of this study. Studies have shown that the tumor microenvironment of peritoneal mesothelioma is rich in immune cells, and the pathological complete response rate of neoadjuvant chemotherapy is high regardless of its subtype. However, the relationship between Ki67 and immune cell infiltration in peritoneal mesothelioma remains unclear. Here, we extensively evaluated Ki67 expression in mesothelioma and its association with the level of immune infiltration. The results showed that the expression of Ki67 was significantly correlated with B cells ($P < .001$, r=0.403), $CD4^+$T cells ($P < .05$, r=0.228), $CD8^+$T cells ($P < .001$, r=0.085), macrophages ($P < .001$, r=0.145), dendritic cells ($P < .001$, r=0.445). In addition, Ki67 expression was correlated with the cumulative survival rate among different immune-infiltrating cells in peritoneal mesothelioma, and patients with high levels of infiltration had poor prognosis. In this study, through GO and KEGG pathway enrichment analysis of Ki67 and its related genes, we speculated that Ki67 may affect the occurrence and development of mesothelioma through these regulatory pathways.

Despite the above study on the value of Ki67 in peritoneal mesothelioma, we still have some limitations. Firstly, most of the data we analyzed were downloaded from online databases and lacked experimental validation in terms of experimental zoology and molecular biology. Secondly, the peritoneal

mesothelioma samples we studied were limited and not representative of the entire peritoneal mesothelioma population, and further validation using techniques such as real-time PCR or Western blot is needed (For example, Westernblot was used to quantitatively analyze the expression level of Ki67 in peritoneal mesothelioma tissues and cell lines, and the results were compared with adjacent tissues and verified by immunohistochemistry). Finally, this study is retrospective and needs to be supported by further prospective findings.

In conclusion, the expression and prognostic significance of Ki67 in peritoneal mesothelioma tissues were analyzed by bioinformatics analysis and validated on clinical specimens. The results showed that asbestos exposure history and Ki67 expression level had important clinical guiding significance for the prognosis of patients with peritoneal mesothelioma.

ETHICAL COMPLIANCE

This study was approved by the ethics committee of Zhuji People's Hospital of Zhengjiang Province. Signed written informed consent were obtained from the patients and/or guardians.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

AUTHOR CONTRIBUTIONS

YD and YZ designed the study and performed the experiments, YZ collected the data, KL analyzed the data, and YD prepared the manuscript. All authors read and approved the final manuscript.

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