Original paper

Comparison of SKIP expression in malignant pleural mesotheliomas with Ki-67 proliferation index and prognostic parameters

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We aimed to determine the presence of SKI-interacting protein (SKIP) expression in malignant pleural mesothelioma (MPM) and its effect on prognosis by investigating SKIP correlation with the Ki-67 proliferation index and prognostic parameters.

Pathological preparations of the patients diagnosed with MPM between 2006 and 2012 were evaluated. Immunohistochemical analyses were performed to evaluate the expression of SKIP and the Ki-67 proliferation index. Correlations between SKIP expression, clinicopathological factors and survival were investigated. Survival data were calculated using the Kaplan–Meier method, and Cox regression analysis was used to evaluate the prognostic value of the variables.

In total, 52 patients were evaluated in the study; 36 of them were male and 16 were female. The mean age of the patients was 62.3 ±12.2 years.

The median overall survival period was 8.5 months. Factors negatively affecting general survival in the univariate analysis included high SKIP expression, Ki-67 proliferative index over 30%, presence of non-epithelioid type MPM and stage III-IV disease ($p < 0.05$).

Cox regression analysis revealed that high SKIP expression, high Ki-67 proliferative index and presence of non-epithelioid type MPM are independent factors that affect the survival rate.

Higher SKIP expression is associated with poor prognosis in MPM.

Key words: mesothelioma, SKI-interacting protein (SKIP), Ki-67, survival.

Introduction

Malignant mesothelioma is a cancer of mesothelial cells mainly originating from the pleura, although the pericardium, peritoneum or tunica vaginalis may also be affected. Malignant pleural mesothelioma (MPM) is related to environmental and occupational asbestos contact and typically has a poor prognosis. Although the most significant etiological agent is asbestos, radiotherapy and inhalation of other silicates, such as thorium dioxide and erionite, are other etiological factors [1, 2].

Despite the therapeutic applications including chemotherapy, radiotherapy, immunotherapy and radical surgery performed in some patients, MPM

still has a poor prognosis, with a reported average survival of approximately 12 months [3, 4, 5].

Different scoring systems have been used to estimate the prognosis of MPM. In these systems, performance conditions, disease subtypes, age, gender and basic laboratory parameters of the patients were used [6, 7]. However, these systems have not been routinely used because of difficulties in evaluating multiple parameters and in applying them to clinical settings. The most important prognostic factors are accepted as histological type, performance condition of the patient and weight loss. Epithelial histology, early tumor stage, high performance score and absence of weight loss are factors positively affecting survival [6, 7].

Various studies have been performed to determine the correlation of MPM progression with markers such as survivin, fibulin-3 and mesothelin [8, 9, 10]. Although a relationship with prognosis and diagnosis was found for some of these markers, it was not found in some others. However, an immunohistochemically detectable marker that accurately defines the prognosis has not been determined yet. Therefore, a cheap, repeatable, effective and easy parameter is required to accurately predict the MPM prognosis.

Ki-67 is a nuclear non-histone protein that can be used as a proliferation marker because its activity increases during mitosis. It may also be used to evaluate the division speed of tumor cells [11]. Ki-67 is a monoclonal antibody developed against a nuclear antigen only available in proliferated cells. Determining Ki-67 expression levels has been demonstrated to be helpful in predicting the prognosis of breast cancer, prostate cancer and many other malignancies such as malign mesothelioma [12, 13, 14].

Ski-interacting protein (SKIP) is a transcriptional cofactor effective in oncogenesis, controlling many signaling pathways that have a role in cell multiplication and differentiation [15, 16]. In the early stages of cancer formation, up-regulation of SKIP protein has been detected. Increased SKIP expression in breast cancer, hepatocellular cancer (HCC) and bladder cancer was found to correlate with a poor prognosis; however, there have so far been no studies investigating the correlation between SKIP expression and the prognosis of MPM [17, 18, 19].

In the present study we aimed to identify the presence of SKIP expression in MPM and to evaluate its relationship with the Ki-67 proliferation index and prognostic parameters.

Material and methods

Local ethical approval was obtained from the Dicle University Medical Faculty Ethics Committee. The medical records and the pathologic blocks of the patients with MPM between 2006 and 2012 were retrospectively evaluated. If the patients met the following criteria, they were excluded from the study: 1) the blocks were taken from the archives for consultation in another centre; 2) insufficient tissue quality and/or quantity in available blocks; 3) patients who were lost to follow-up. Fifty-two formalin-fixed paraffin-embedded tumor tissue samples and slides were retrieved in total from the archives of the Pathology Department, with relevant clinical data (gender, age at diagnosis and follow-up data) obtained from the patient records.

Diagnostic re-evaluation of the slides of each case was made according to the World Health Organization classification guidelines of MPM (epithelioid, sarcomatoid, desmoplastic, or biphasic). The clinical records and histopathological diagnosis of all patients were fully documented.

All cases were clinically staged in accordance with the tumor, node, metastasis (TNM) classification of MPM.

Age (≤ 60 or > 60 years old), gender, histopathological subtype (epithelial or non-epithelial), stage of the disease (stage I-II or stage III-IV), low (score of 1) or high (score of 2 or 3) SKIP expression and Ki-67 proliferation index ($\leq 30\%$ or $> 30\%$) were determined as evaluation criteria.

Immunohistochemistry

Ki-67 (pre-diluted, ready-to-use; PRM 325 AA, rabbit monoclonal, Biocare, Concord, USA) and anti-SKIP antibodies (1 : 200 dilution; ab154575, rabbit polyclonal, Abcam, Cambridge, UK) were used for immunohistochemical staining. After the archive case slides were re-examined by light microscopy, 4-μm cross-sections were made from the paraffin blocks. The tissue sections were stained by standard immunohistochemical techniques using a Ventana BenchMark Ultra Automated Immunostainer (BenchMark Ultra; Ventana Medical Systems, Tucson, AZ, USA), using heat-mediated antigen retrieval with citrate buffer at pH 6 and a standard diaminobenzidine detection kit (Ventana). Evaluations were made by light microscopy (Olympus BX53, Tokyo, Japan).

Immunostained sections were examined by two investigators (G.T and U.A.) who were blind to patient characteristics. Nuclear staining was accepted as Ki-67 positivity. Approximately 500 cells were counted on every slide, and the evaluation of proliferative activity (proliferation index) was made according to the study by Deraco *et al*. [20].

The SKIP expression was assessed by semi-quantitative (manual) scoring. Only cytoplasmic staining was adopted. The presence of SKIP staining in 10% or more of tumor cells was accepted as SKIP positivity. Intensity of staining was graded as weak (score of 1), moderate (score of 2), or strong (score of 3).

Statistical analysis

SPSS version 11.5 (Statistical Package for the Social Sciences) was used for statistical analyses. The correlation of SKIP expression with the patients' clinical and pathological characteristics was analyzed using the chi-square test. The effects of clinical and pathological characteristics and SKIP expression status on general survival were evaluated by the Kaplan–Meier method in univariate analysis and by the Cox regression method in multivariate analysis. Results were considered statistically significant at a p value less than 0.05.

Results

We reviewed the medical records of 89 patients with MPM. Of them, 52 patients were eligible for the study. The mean age of the patients was 62.3 ±12.2 years (40-83 years); 36 (69.2%) of them were male and 16 (30.8%) were female. The distribution of MPM histological types was as follows: 45 epithelioid (86.5%), 4 sarcomatoid (7.7%) and 3 biphasic (5.8%).

Immunohistochemistry

SKIP and Ki-67 expression was observed in all MPM tissues in our study (Figs. 1 and 2). SKIP staining was observed in a cytoplasmic expression pattern in these tissues. Nuclear staining was observed with Ki-67. The scoring of SKIP expression showed high levels of positivity in 21 cases and low levels of it in 31 cases. The Ki-67 proliferation index was greater than 30% in 18 cases and equal to or lower than 30% in 34 cases.

There were significant differences in the expression of SKIP and the Ki-67 proliferation index in the epithelioid and non-epithelioid histological types (Table I).

Survival

The overall median survival of the patients was 8.5 months (95% CI: 9.37-15.13).

Clinical and pathological characteristics were evaluated by univariate and multivariate analyses. According to the univariate log rank test, the survival period was found to be significantly shorter ($p < 0.05$)

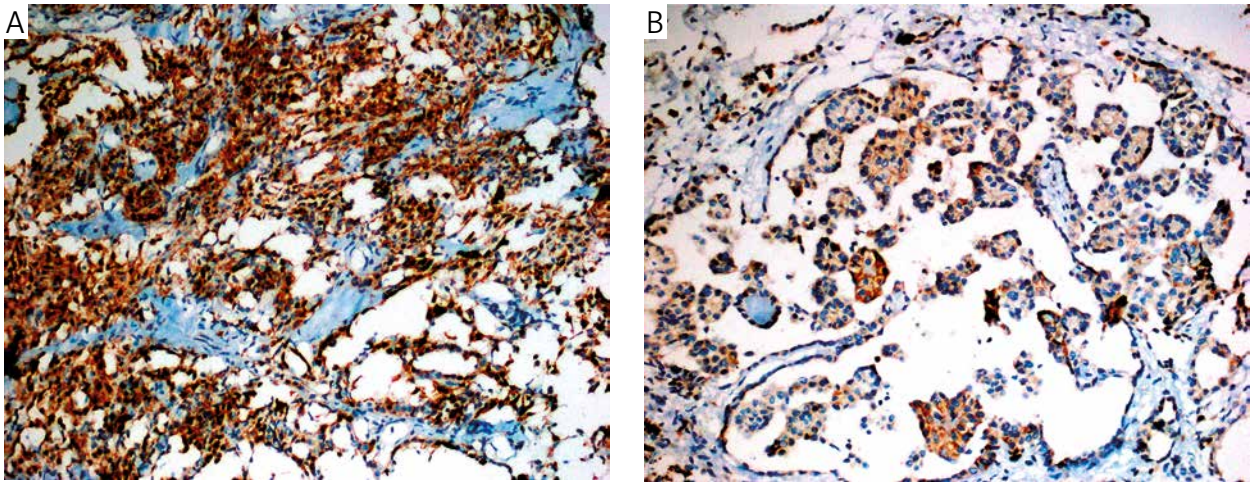

Fig. 1. High (A) and low (B) SKIP expression in MPM cells

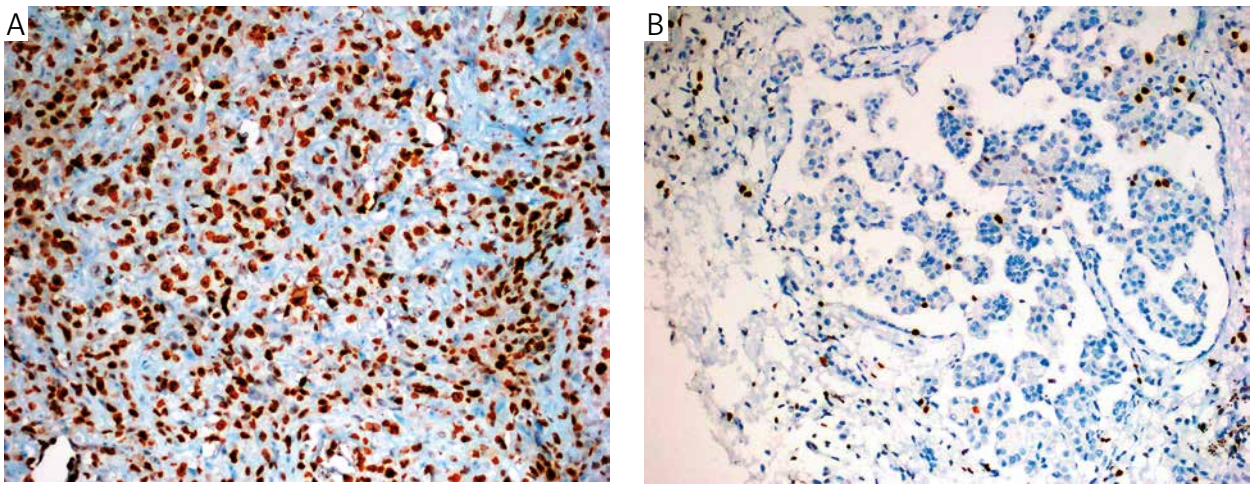

Fig. 2. Nuclear Ki-67 positivity is seen in many of the MPM cells in (A) and in a few of them in (B)

Table I. Survival status and clinicopathological parameters in 52 malignant pleural mesothelioma patients

Parameters	SKIP		p	Ki-67		p
	Low	High		Low	High	
Sex						
Male	21	15	0.512	24	12	0.504
Female	10	6		10	6	
Age (years)						
≤ 60	17	9	0.286	18	8	0.386
> 60	14	12		16	10	
Stage						
Stage I-II	0	4	0.022	4	0	0.171
Stage III-IV	17	31		30	18	
Tumor types						
Epithelioid	25	20	0.038	32	13	0.041
Non-epithelioid	2	5		2	5	
Ki-67						
Low	16	18	0.011			
High	15	3				

in relation with the Ki-67 proliferation index being over 30%, increased SKIP expression intensity, presence of non-epithelial histological type of MPM and the disease stage being III or IV during diagnosis (Table I). Figures 3 and 4 show the Kaplan–Meier survival curves of the patients compared with SKIP and Ki-67 expression. There was no effect of age or gender on the prognosis of MPM. In the multivariate analysis, while it was determined that SKIP expression prevalence, Ki-67 proliferation index being over 30% and non-epithelial histological type had an independent negative effect on survival according to the multivariable Cox regression analysis results, there could not be detected any effect of advanced stage disease as an independent factor on survival. When the effects on survival were examined in Cox regression analysis, it was observed that high SKIP expression intensity, a Ki-67 proliferation index over 30% and non-epithelioid histological type of MPM reduced survival by two-fold, four-fold and five-fold respectively (Table II).

A Ki-67 proliferation index below 30% was found 15.5 ±1.9 years, being over 30% was found 6.2 ±0.9 years, 12.3 ±1.4 years in total, in those with lower SKIP expression 20.5 ±2.2 years, in those with high SKIP expression 6.6 ±1.0 years, 12.3 ±1.4 years, in epithelioid type 13.3 ±1.6 years, in non-epithelioid type as 5.7 ±1.7 years and 12.3 ±1.4 years in total.

A moderate positive correlation was observed between the Ki-67 proliferation index and SKIP expression ($p = 0.011$, $r = 0.352$).

Discussion

To the best of our knowledge, this is the first study to evaluate the effect of SKIP expression on prognosis in MPM patients. We found that SKIP expression may be an indicator of poor prognosis in MPM patients. However, this study was a preliminary investigation, with a small sample size; therefore, a larger study is warranted to confirm the results.

Malignant mesothelioma has a poor prognosis and occurs due to environmental factors. Like other types of malignancy, it is a disease with a complex, heterogeneous genetic and biochemical background. The exact mechanisms of MPM pathogenesis are currently unclear [1, 2, 3, 4, 5]. The multifarious biological and clinical features of MPM have prompted the search for more useful prognostic and predictive markers for use in diagnosis and therapy.

Notch signaling proteins, SKIP, tumor growth factor-β signaling proteins, CBF-1, Smad2 and E7 oncoprotein are transcriptional cofactors that play roles in many signaling pathways that control cell multiplication and differentiation; thus, they may also be involved in oncogenesis [15, 16, 21]. Increased SKIP protein is detected in early cancer formation, and SKIP expression has been described as a promising factor for predicting prognosis in HCC, breast cancers and bladder cancers [17, 18, 19]. The present study also indicates SKIP expression to be useful for predicting poor outcome in patients with MPM. Biomarkers such as survivin, fibulin-3 and mesothelin have also been suggested as prognostic factors to

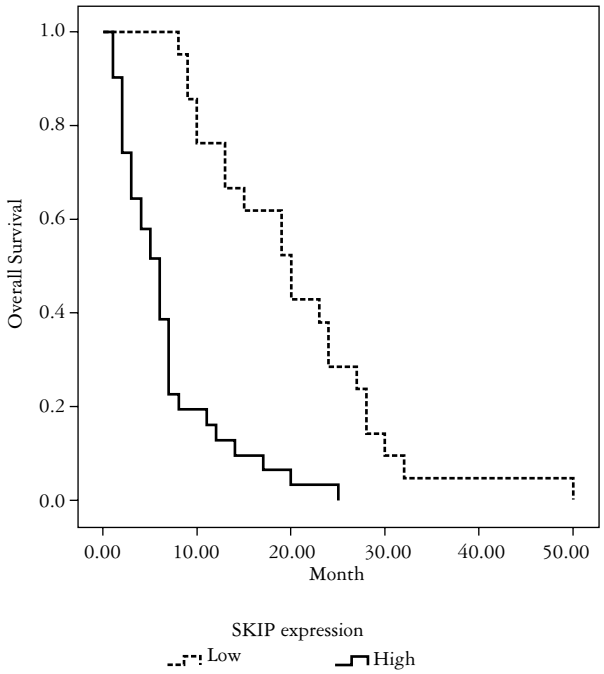

Fig. 3. Kaplan–Meier survival curves of patients compared with SKIP expression

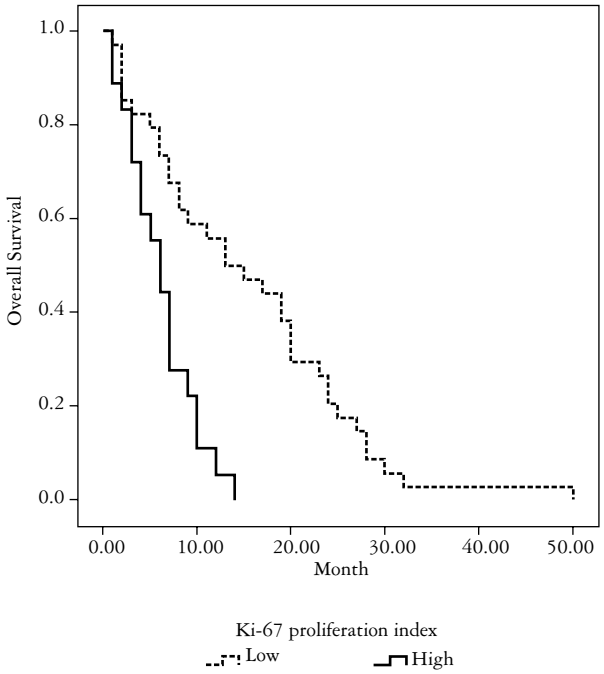

Fig. 4. Kaplan–Meier survival curves of patients compared with Ki-67 proliferation index

Table II. Multivariate Cox regression analysis of overall survival in patients with malignant pleural mesotheliomas

Prognostic variables	Hazard ratio	95% confidence interval	p
Age (years)	11.05	0.99-1.01	0.108
Sex	0.52	0.18-1.818	0.306
Stage	0.65	0.63-2.26	0.152
Histological type	2.39	1.48-4.45	0.041
Ki-67	4.56	2.25-9.25	0.03
SKIP	2.46	1.23-4.93	< 0.001

predict poor outcome in MPM, but they are not sufficient for use as prognostic biomarkers [8, 9, 10].

It is known that the Ki-67 proliferation index increases in MPM patients and that it is related to poor prognosis [9, 14]. In HCC, alterations in SKIP expression and the Ki-67 proliferation index were found to be correlated, and it was reported that the increase of both of them is a short survey indicator in HCC patients [18]. In our study, an increase in SKIP expression and the Ki-67 proliferation index was detected in MPM patients, and it was observed that the increase in these values was related to poor prognoses. It was demonstrated that the increase in Cox regression analysis reduced the survival rate two-fold.

In HCC patients, it was found that SKIP was effective in proliferation assessment similar to Ki-67. It has been reported that proliferation of SKIP expressing cells in HCC increases in G1 and S phases of the cell cycle [18]. It was also found that SKIP expressed by the retinoblastoma gene directly affects pRb protein. It was suggested that SKIP could advance tumorigenesis by being effective in cell proliferation [16]. The presence of a correlation between both values in our study suggests that SKIP is effective in proliferation assessment in MPM patients, similarly to the case in HCC.

Many factors were defined regarding the prognosis of MPM patients. These included age, gender, non-epithelioid histological type, thrombocytosis and different biological agents [2, 22]. We did not find any effect of age or gender on the prognosis of MPM. In the multivariate Cox regression analysis, it was found that the factors affecting survival were SKIP expression intensity, Ki-67 proliferation index and non-epithelioid histological type. In previous studies, it was reported that the Ki-67 proliferation index was

higher in MPM cases and that survival periods were shorter [9, 14]. Similarly, the Ki-67 proliferation index was higher in non-epithelioid cases in our study.

SKIP expression has not been studied before in MPM cases, but it has been reported as a prognostic factor in HCC, cancer of the bladder or breast [17, 18, 19]. It has been reported that in high-grade urothelial carcinoma, SKIP mRNA levels increase much more than those in low-grade urothelial carcinoma and that this is associated with a decrease in the survival rate [19]. In breast cancer, it was reported that the increase in SKIP expression was correlated with an increase in the Ki-67 proliferative index [17]. An increase in SKIP expression in the G1 and S phases was also reported. Additionally, it was observed that the increase in SKIP expression caused a decrease in p27 values in MCF-7 and MDA-MB-231 cell lines, which resulted in oncogenic potential. In breast cancer cases, it was detected that the increase in SKIP value was related to lower patient survival [16, 17, 18].

In conclusion, we observed that SKIP expression was increased in MPM patients. The increase in SKIP expression was found to correlate with the Ki-67 proliferative index, and it is an effective factor predicting the patient survival period. Although this was a preliminary study, we identified SKIP expression as a useful predictor of poor prognosis. Clinical studies investigating this approach will be required to confirm our results.

The authors declare no conflict of interest.

References

1. Gibbs AR. Role of asbestos and other fibres in the development of diffuse malignant mesothelioma. Thorax 1990; 45: 649-654.
2. O'Reilly KM, McLaughin AM, Beckett WS, et al. Asbestos related lung disease. Am Fam Physician 2007; 75: 683-688.
3. Montanaro F, Rosato R, Gangemi M, et al. Survival of pleural malignant mesothelioma in Italy: A populationbased study. Int J Cancer 2009; 124: 201-207.
4. Borasio P, Berruti A, Billé A, et al. Malignant pleural mesothelioma: Clinicopathologic and survival characteristics in a consecutive series of 394 patients. Eur J Cardiothorac Surg 2008; 33: 307-313.
5. Batirel HF, Metintas M, Caglar HB, et al. Trimodality treatment of malignant pleural mesothelioma. J Thorac Oncol 2008; 3: 499-504.
6. Curran D, Sahmoud T, Therasse P, et al. Prognostic factors in patients with pleural mesothelioma: the European Organization for Research and Treatment of Cancer experience. J Clin Oncol 1998; 16: 145-152.
7. Herndon JE, Green MR, Chahinian AP, et al. Factors predictive of survival among 337 patients with mesothelioma treated between 1984 and 1994 by the cancer and Leukemia Group B. Chest 1998; 113: 723-731.
8. Kirschner MB, Pulford E, Hoda MA, et al. Fibulin-3 levels in malignant pleural mesothelioma are associated with prognosis but not diagnosis. Br J Cancer 2015; 113: 963-969.
9. Hmeljak J, Erculj N, Dolžan V, et al. Is survivin expression prognostic or predictive in malignant pleural mesothelioma? Virchows Arch 2013; 462: 315-321.
10. Righi L, Cavallo MC, Gatti G, et al. Tumor/stromal caveolin-1 expression patterns in pleural mesothelioma define a subgroup of the epithelial histotype with poorer prognosis. Am J Clin Pathol 2014; 141: 816-827.
11. Gerdes J, Li L, Schlueter C, et al. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol 1991; 138: 867-873.
12. Berney DM, Gopalan A, Kudahetti S, et al. Ki-67 and outcome in clinically localised prostate cancer: analysis of conservatively treated prostate cancer patients from the Trans-Atlantic Prostate Group study. Br J Cancer 2009; 100: 888-893.
13. Rossi L, Laas E, Mallon P, et al. Prognostic impact of discrepant Ki67 and mitotic index on hormone receptor-positive, HER2-negative breast carcinoma. Br J Cancer 2015; 113: 996-1002.
14. Kobayashi M, Huang CL, Sonobe M, et al. Intratumoral Wnt2B expression affects tumor proliferation and survival in malignant pleural mesothelioma patients. Exp Ther Med 2012; 3: 952-958.
15. Dahl R, Wani B, Hayman MJ. The Ski oncoprotein interacts with Skip, the human homolog of Drosophila Bx42. Oncogene 1998; 16: 1579-1586.
16. Prathapam T, Kuhne C, Banks L. Skip interacts with the retinoblastoma tumor suppressor and inhibits its transcriptional repression activity. Nucleic Acids Res 2002; 30: 5261-5268.
17. Liu X, Ni Q, Xu J, et al. Expression and prognostic role of SKIP in human breast carcinoma. J Mol Histol 2014; 45: 169-180.
18. Liu G, Huang X, Cui X, et al. High SKIP expression is correlated with poor prognosis and cell proliferation of hepatocellular carcinoma. Med Oncol 2013; 30: 537.
19. Wang L, Zhang M, Wu Y, et al. SKIP expression is correlated with clinical prognosis in patients with bladder cancer. Int J Clin Exp Pathol 2014; 7: 1695-1701.
20. Deraco M, Cabras A, Baratti D, et al. Immunohistochemical evaluation of minichromosome maintenance protein 7 (MCM7), topoisomerase IIα, and Ki-67 in malignant peritoneal diffuse mesothelioma patients using tissue microarray. Ann Surg Oncol 2015; 22: 4344-4351.
21. Leong GM, Subramaniam N, Figueroa J, et al. Ski-interacting protein interacts with Smad proteins to augment transforming growth factor-beta-dependent transcription. J Biol Chem 2001; 276: 18243-18248.
22. Edwards JG, Abrams KR, Leverment JN, et al. Prognostic factors for malignant mesothelioma in 142 patients: validation of CALGB and EORTC prognostic scoring systems. Thorax 2000; 55: 731-735.

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