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Clinicopathological Significance of Expression of Tspan-1, Jab1 and p27 in Human Hepatocellular Carcinoma

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The aim of this study was to investigate the expression of Tspan-1, Jab1 and p27 in human hepatocellular carcinoma (HCC) and their clinicopathological significance. The expression of Tspan-1, Jab1 and p27 was detected in HCC tissues, the tissues around cancer (76 cases), and the normal tissues around the liver hemangiomas (10 cases). The overexpression of Tspan-1 and Jab1 was found in HCC tissues, positively correlated with clinical stage and negatively correlated with survival rate. The expression of p27 was found inversely linked to which of Tspan-1 and Jab1. In conclusion, the expression of Tspan-1, Jab1 and p27 is significantly associated with development of HCC. Overexpression of Tspan-1 and Jab1 suggests poor prognosis but overexpression of p27 may expect good prognosis for patients with HCC.

Key Words: Carcinoma, Hepatocellular; Tspan-1; Jab1; p27; Prognosis

INTRODUCTION

Survival rate of the patients with hepatocellular earcinoma (HCC) was increased due to the advancement of diagnosis and treatment, however, the total tendency has yet to be improved. Most cases were diagnosed at late stages. So far, the rate of surgical resection for HCC is around 20%; for most patients surgical treatment was not indicated (1). A previous study reported that the five-year survival rate after radical resection was less than 50%, and the rate of metastasis was 61.5% (2).

Tetraspanins is a large family of ubiquitously expressed membrane proteins. Several tetraspanins molecules such as CD9, CD82, CD63 and CD151 have been identified and implicated in the regulation of cell development, differentiation, proliferation, motility and tumor cell invasion (3-7). Tspan-1 (or NET-1, Gene ID: 10103) is a new member of the tetraspanins group. Sequence analysis of Tspan-1 revealed a structure typical for tetraspanins, with the presence of four transmembrane domains delimiting two extracellular regions as well as conserved amino acid residues (8). Tspan-1 was found to be overexpressed in some tumors (9-14). Studies reported Tspan-1 expression may be associated with tumor cells proliferation (9, 15). All of these reports suggest that Tspan-1 may play a critical role in the progression of tumor growth and metastasis in numerous human tumors, including HCC.

P27 is a well known negative regulator of cell cycle progression. Jab1 directly binds to p27 and induces nuclear export and subsequent degradation (16). Some reports have shown that overexpression of Jab1 and low expression of p27 is associated with advanced tumor stage and poor prognosis in several human cancers (16-19), including HCC (20).

In the present study, we examined the expression of Tspan-1 combined with Jab1, and p27 in specimens of 76 patients with HCC. The relationship between the expression of Tspan-1, Jab1 and p27 with the clinicopathological factors and the prognosis of HCC patients were explored.

MATERIALS AND METHODS

Clinical data of the patients

Seventy-six specimens in this study were obtained from the patients with HCC in our hospital undergoing surgical operations of the liver during 1998-2004. The number patients was 76 cases (male 60, female 16) and mean age was 58 yr old (from 35 to 79). Histologically 10 cases were well differentiated, 42 cases were intermediate differentiated, and 24 cases were poorly differentiated. Of them 23 cases had portal vein tumor thrombus and distant dissection. Normal control tissues of the liver were tak-

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en from ten patients operated for hemangioma. The follow-up period of the patients exceeded 60 months. Informed consent was obtained before any specimens were taken. The study protocol was approved by the Ethics Committee of the affiliated hospital of Nantong University (NTU090024).

Antibodies used in immunohistochemisty

The antibodies used for immunohistochemistry in this study were: Tspan-1 rabbit anti-human polyclonal antibody (1:100; devised by the author and prepared under the cooperation of the American San Francisco Gene Biological Company, San Francisco, CA, USA), mouse monoclonal antibody against human Jab1 (611618, 1:50;BD Biosciences PharMingen, San Diego, CA, USA), rabbit anti-human p27 polyclonal antibody (C-19, 1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), HRP-goat anti-rabbit IgG (1:200; Sigma-Aldrich, St. Louis, MO, USA), HRP-goat anti-mouse IgG (1:200; Sigma-Aldrich, St. Louis, MO, USA).

Immunohistochemical examination of expression of Tspan-1, Jab1 and p27

Two-step immunohistochemical method was performed on formalin-fixed, paraffin-embedded 5-μm sections from all patients to detect the expression of Tspan-1, Jab1 and p27 in HCC. Five consecutive slides were prepared from each tissue. The slides were deparaffinized by dimethyl benzene, dehydrated by gradient ethanol. For antigen retrieval, they were heated at 95°C for 10 min in sodium citrate buffer (10 mM sodium-citrate monohydrate, pH 6.0). Slides were allowed to cool for 20 min at room temperature, incubated in 0.3% H_2O_2 at room temperature for 15 min to inhibit endogenous peroxidase, and then incubated with primary antibodies overnight at 4°C. Two-step reagent kit (HRP- anti-mouse/rabbit IgG) was then applied to detect the immunoreactivity. Slides were stained by DAB, and counterstained by hematoxylin. Rabbit IgG was used to replace the primary antibody as control staining. Gastric cancer tissue was used as positive control.

For microscopy, five random high-magnification fields (×400) were selected from each slide to count 1,500 tumor cells. Percentage of positive cells was calculated and recorded as Label Index (LI=% of positive cells). The mean value of LI of Tspan-1, Jab1 and p27 (0.70, 0.69 and 0.26, respectively) in HCC tissues was used to define the over and low expression according to a previous study by other author (21).

Statistical analysis

The data of the expression of the proteins are presented as means ±SD. Spearman test was applied to detect the correlation of among Tspan-1, Jab1 and p27 expression. Fisher's exact test was used to compare the expression of all proteins as groups (positive vs. negative) with various clinicopathological parameters. Survival analysis was undertaken using Kaplan-Meier method and group differences in survival time were investigated by log-rank test. The multiple factor survival analysis was evaluated using Cox's proportional hazards model. *P* values less than 0.05 was considered statistically significant. SPSS for Windows (version 13.0, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

RESULTS

Expression of Tspan-1, Jab1 and p27 in HCC

We studied the expression of Tspan-1, Jab1 and p27 in HCC tissues (76 cases), the tissues around HCC (76 cases), and the normal tissues around the liver hemangiomas (10 cases). The expression of Tspan-1 in HCC cells varies from cytoplasm to membrane, or mixed. Jab1 and p27 were mainly expressed in nucleus, but also in cytoplasm (Fig. 1). The difference of expression levels of these molecules in the three types of tissues was shown in Table 1. Statistical analysis revealed that the expression of Tspan-1, Jab1 was significantly higher in HCC than in the tissues around cancer or normal control ($P<0.05$). And the expression of p27 in HCC was remarkably lower than in the other two tissues ($P<0.05$).

Correlation of the expression of Tspan-1, Jab1 and p27 in HCC

The correlation of expressions of Tspan-1, Jab1 and p27 in HCC was examined by Spearman test. Statistical analysis showed

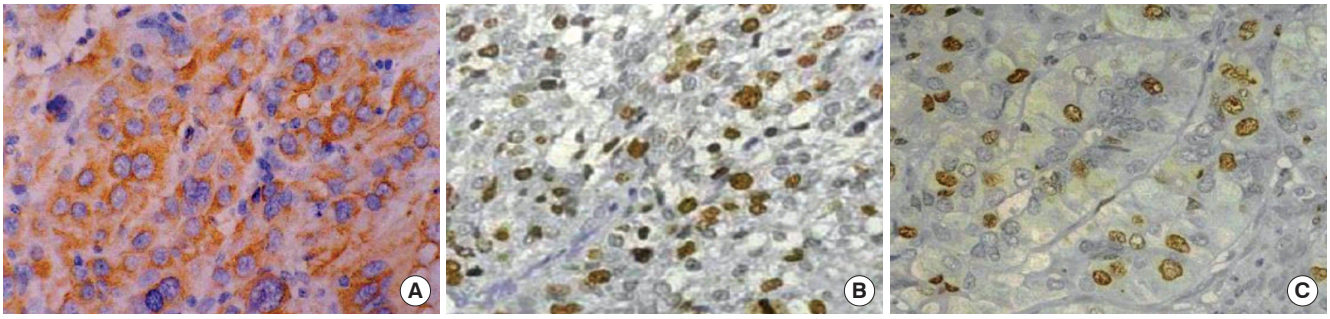

Fig. 1. Immunostaining of Tspan-1, Jab1 and p27 in HCC. (**A**) Tspan-1 expression, indicated by yellowish granules, varies from cytoplasm to membrane, or mixed. (**B**) Jab1 and (**C**) p27 expression, indicated by brownish yellow granules, are mainly in nuclear, but also can be found in cytoplasm. Magnification ×200.

that Tspan-1 was positively associated with Jab1 (r=0.6045, $P<0.001$), but inversely correlated with p27 (r=-0.6971, $P<0.001$). It also indicated that Jab1 was inversely associated with p27 (r=-0.7089, $P<0.001$).

Table 1. Expression of Tspan-1, Jab1 and p27 in HCC tissues, the tissues around cancer and the normal tissues (%)

Proteins	The normal tissues (A)		Tissues around cancer(B)		HCC tissues (C)	
	LI	P^1	LI	P^2	LI	P^3
Tspan-1	10.68±1.76	<0.001	36.87±9.77	<0.05	69.65±14.06	<0.001
Jab1	34.36±8.16	<0.001	38.72±9.56	>0.05	68.85±12.10	<0.001
p27	73.58±10.44	<0.001	76.20±8.20	>0.05	26.50±13.30	<0.001

P^1, A group vs. C; P^2, A group vs. B; P^3, B group vs. C; LI, % of positive cells.

Table 2. Correlation of the expression of Tspan-1, Jab1 and p27 with clinicopathological factors in HCC

Clinical parameter	Samples	Tspan-1		*P*	Jab1		*P*	p27		*P*
		Low ≤0.70	Over >0.70		Low ≤0.69	Over >0.69		Low ≤0.26	Over >0.26	
Age (yr)										
≤45	21	8	13		7	14		13	8	
>45	55	26	29	0.472	26	29	0.273	31	24	0.662
Gender										
Male	60	29	31		27	33		34	26	
Female	16	5	11	0.222	6	10	0.591	10	6	0.675
Differentiation										
Well	10	6	4		8	2		2	8	
Intermediate	42	24	18		20	22		20	22	
Poor	24	4	20	0.003	5	19	0.005	22	2	0
Metastasis										
N1-3	23	7	16		5	18		20	3	
N0	53	32	21	0.016	28	25	0.012	24	29	0.001

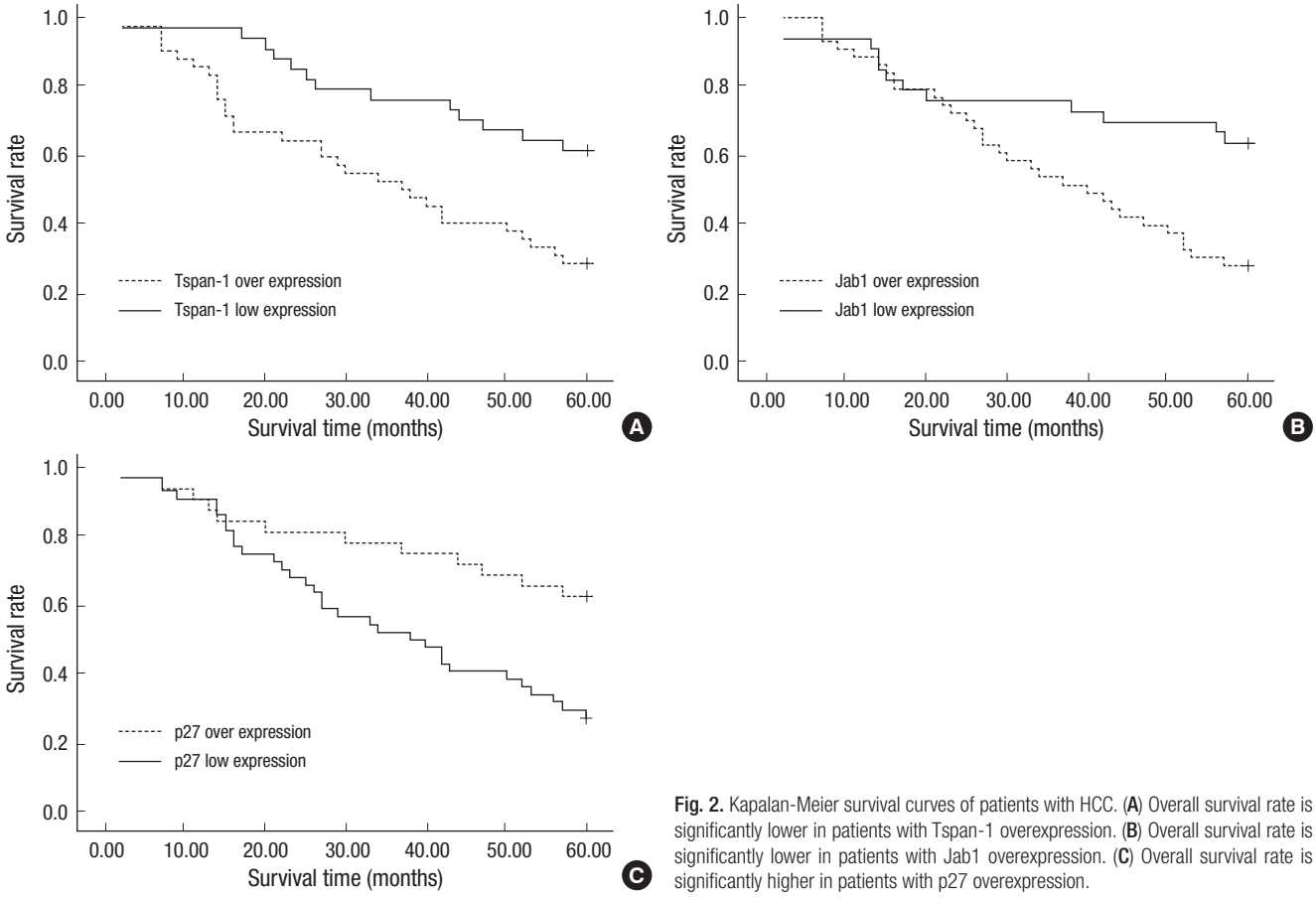

Fig. 2. Kapalan-Meier survival curves of patients with HCC. (**A**) Overall survival rate is significantly lower in patients with Tspan-1 overexpression. (**B**) Overall survival rate is significantly lower in patients with Jab1 overexpression. (**C**) Overall survival rate is significantly higher in patients with p27 overexpression.

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Correlation of the expression of Tspan-1, Jab1 and p27 with clinicopathological factors in HCC

The overexpression of Tspan-1, Jab1 was negatively related to cancer cell differentiation and tumor metastasis (P<0.05). The overexpression of p27 was significantly and positively related to cancer differentiation and tumor metastasis (P<0.05). There was no significantly difference between low expression and over expression of the three proteins in terms of age and gender (P> 0.05) (Table 2).

Correlation of the expression of Tspan-1, Jab1 and p27 with the patients' survival

Survival analysis was done in 76 patients in whom follow-up data and results for the expression of Tspan-1, Jab1 and p27 were available. By using the Kaplan-Meier analysis, the patients with Tspan-1 and Jab1 overexprssion are significantly associated with short overall survival (P<0.05) (Fig. 2A, B). Statistical analysis also indicated that the survival rate of the p27 overexpression group was significantly higher than that of the low expression group (P<0.05) (Fig. 2 C). Multivariate analysis using the Cox's proportional hazards model showed that Tspan-1, Jab1 and p27 protein are independent prognostic indicators for patients' overall survival (P=0.018, P=0.012, P=0.002, respectively).

DISCUSSION

Tspan-1, a new member of the tetraspanins group, is a recently discovered tumor-related gene. Some reports had indicated that Tspan-1 can stimulate proliferation, invasion and motility of tumor cells (9, 22). In the present study, we found that the expression of Tspan-1 in HCC tissues was significantly higher than the tissues around cancer or the normal tissues. There is also a strong positive correlation between the level of Tspan-1 expression and degree of HCC cell differentiation and clinical stages. Furthermore, we found that Tspan-1 was an independent factor affecting prognosis of HCC. The over expression of Tspan-1 increased the risk of death. Five-year survival rate in patients with overexpression of Tspan-1 was significantly lower than those patients with low expression of Tspan-1.

In the present study, we found that Jab1 and p27 expression were inversely correlated, and significantly associated with malignancy, prognosis and survival rate in patients with HCC. Similar to our work, other reports indicated that Jab1 overexpression was significantly associated with poor prognosis of patients with tumors such as ovarian tumor, lung cancer and breast cancer, and was inversely associated with p27 (23-25). It has been known that p27 regulate cell proliferation as Cdk inhibitor by inhibiting cell cycle progression from G1 to S phase in a dose-dependent fashion. Jab1 can accelerate the degradation of p27 by translocating p27 from nucleus to cytoplasm where degradation occur (26).

The expression of Tspan-1 in cancer cells displayed cytoplasmic or membranous patterns, which showed the distribution and functional sites of the Tspan-1 molecule in cells. The molecule may accept extracellular signals when located on the membrane and carry out functions in the cytoplasm, like other tetraspanins such as CD9, CD82 and CD63 (27, 28). Leyden et al. reported that siRNA-mediated downregulation of Tspan-1 inhibited the proliferation of gastric cancer cells in vitro by inhibiting cell cycle progression from G1 to S phase (9). In our study, we found that Tspan-1 was positively associated with Jab1, and inversely correlated with p27. It has been known that p27 was degraded specifically in the cytoplasm by the ubiquitin (Ub)-proteasome system (29). So we pursued the question whether Tspan-1 interacted with some other proteins to affect the ubiquitin (Ub)-proteasome system degrading p27 or interacted with Jab1 to accelerate the transportion of p27 from nucleus to cytoplasm, and then led to accelerate the tumor cells proliferation.

In summary, the expression of Tspan-1, Jab1 and p27 in HCC is involved in cancer cell behavior including proliferation, differentiation, metastasis, and clinical stage. Overexpression of Tspan-1 and Jab1 suggested poor prognosis but of p27 may expect good prognosis for patients with HCC.

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REFERENCES

1. Chen HB, Huang Y, Dai DL, Zhang X, Huang ZW, Zhang QK, Wang HH, Zhang JS, Pan G. *Therapeutic effect of transcatheter arterial chemoembolization and percutaneous injection of acetic acids on primary liver cancer. Hepatobiliary Pancreat Dis Int 2004; 3: 55-7.*
2. Tang ZY, Ye SL, Liu YK, Qin LX, Sun HC, Ye QH, Wang L, Zhou J, Qiu SJ, Li Y, Ji XN, Liu H, Xia JL, Wu ZQ, Fan J, Ma ZC, Zhou XD, Lin ZY, Liu KD. *A decade's studies on metastasis of hepatocellular carcinoma. J Cancer Res Clin Oncol 2004; 130: 187-96.*
3. Mazzocca A, Carloni V, Sciammetta S, Cordella C, Pantaleo P, Caldini A, Gentilini P, Pinzani M. *Expression of transmembrane 4 superfamily (TM4SF) proteins and their role in hepatic stellate cell motility and wound healing migration. J Hepatol 2002; 37: 322-30.*
4. Furuya M, Kato H, Nishimura N, Ishiwata I, Ikeda H, Ito R, Yoshiki T, Ishikura H. *Down-regulation of CD9 in human ovarian carcinoma cell might contribute to peritoneal dissemination: Morphologic alteration and reduced expression of beta 1 integrin subsets. Cancer Res 2005; 65: 2617-25.*
5. Chen Z, Mustafa T, Trojanowicz B, Brauckhoff M, Gimm O, Schmutzler C, Kohrle J, Holzhausen HJ, Kehlen A, Klonisch T, Finke R, Dralle H, Hoang-Vu C. *CD82, and CD63 in thyroid cancer. Int J Mol Med 2004; 14: 517-27.*
6. Odintsova E, Berditchevski F. *Role of the metastasis suppressor tetraspanin CD82/KAI 1 in regulation of signalling in breast cancer cells. Breast Can-*

cer Res 2006; 8 (Suppl 2): P21.

7. Klosek SK, Nakashiro K, Hara S, Goda H, Hasegawa H, Hamakawa H. *CD151 regulates HGF-stimulated morphogenesis of human breast cancer cells. Biochem Biophys Res Commun 2009; 379: 1097-100.*
8. Serru V, Dessen P, Boucheix C, Rubinstein E. *Sequence and expression of seven new tetraspans. Biochim Biophys Acta 2000; 1478: 159-63.*
9. Leyden J, Murray D, Moss A, Arumuguma M, Doyle E, McEntee G, O'Keane C, Doran P, MacMathuna P. *Net1 and Myeov: computationally identified mediators of gastric cancer. Br J Cancer 2006; 94: 1204-12.*
10. Wollscheid V, Kuhne-Heid R, Stein I, Jansen L, Kollner S, Schneider A, Durst M. *Identification of a new proliferation-associated protein NET-1/C4.8 characteristic for a subset of high-grade cervical intraepithelial neoplasia and cervical carcinomas. Int J Cancer 2002; 99: 771-5.*
11. Chen L, Wang Z, Zhan X, Li DC, Zhu YY, Zhu J. *Association of NET-1 gene expression with human hepatocellular carcinoma. Int J Surg Pathol 2007; 15: 346-53.*
12. Scholz CJ, Kurzeder C, Koretz K, Windisch J, Kreienberg R, Sauer G, Deissler H. *Tspan-1 is a tetraspanin preferentially expressed by mucinous and endometrioid subtypes of human ovarian carcinomas. Cancer Lett 2009; 275: 198-203.*
13. Chen L, Li X, Wang GL, Wang Y, Zhu YY, Zhu J. *Clinicopathological significance of overexpression of TSPAN1, Ki67 and CD34 in gastric carcinoma. Tumori 2008; 94: 531-8.*
14. Chen L, Zhu YY, Zhang XJ, Wang GL, Li XY, He S, Zhang JB, Zhu JW. *TSPAN1 protein expression: a significant prognostic indicator for patients with colorectal adenocarcinoma. World J Gastroenterol 2009; 15: 2270-6.*
15. Wollscheid V, Kuhne-Heid R, Stein I, Jansen L, Kollner S, Schneider A, Durst M. *Identification of a new proliferation-associated protein NET-1/C4.8 characteristic for a subset of high-grade cervical intraepithelial neoplasia and cervical carcinomas. Int J Cancer 2002; 99: 771-5.*
16. Ahn J, Hong SA, Lee SE, Kim J, Oh YS, Park SJ, Chung YJ. *Cytoplasmic localization of Jab1 and p27 Kip1 might be associated with invasiveness of papillary thyroid carcinoma. Endocr J 2009; 56: 707-13.*
17. Esteva FJ, Sahin AA, Rassidakis GZ, Yuan LX, Smith TL, Yang Y, Gilcrease MZ, Cristofanilli M, Nahta R, Pusztai L, Claret FX. *Jun activation domain binding protein 1 expression is associated with low p27(Kip1)levels in node-negative breast cancer. Clin Cancer Res 2003; 9: 5652-9.*
18. Goto A, Niki T, Moriyama S, Funata N, Moriyama H, Nishimura Y, Tsuchida R, Kato JY, Fukayama M. *Immunohistochemical study of Skp2 and Jab1, two key molecules in the degradation of P27, in lung adenocarcinoma. Pathol Int 2004; 54: 675-81.*
19. Korbonits M, Chahal HS, Kaltsas G, Jordan S, Urmanova Y, Khalimova Z, Harris PE, Farrell WE, Claret FX, Grossman AB. *Expression of phosphorylated p27(Kip1) protein and Jun activation domain-binding protein 1 in human pituitary tumors. J Clin Endocrinol Metab 2002; 87: 2635-43.*
20. Lu MD, Wang Y, Chen L, Qin J, Li P, Cui XP, Shen AG. *Expression and significance of Ser10 phosphorylated p27(kip1) and JAB1 protein in human hepatocellular carcinoma. Zhonghua Bing Li Xue Za Zhi 2007; 36: 840-1.*
21. Xu X, Yamamoto H, Sakon M, Yasui M, Ngan CY, Fukunaga H, Morita T, Ogawa M, Nagano H, Nakamori S, Sekimoto M, Matsuura N, Monden M. *Overexpression of CDC25A phosphatase is associated with hypergrowth activity and poor prognosis of human hepatocellular carcinomas. Clin Cancer Res 2003; 9: 1764-72.*
22. Murray D, Horgan G, Macmathuna P, Doran P. *NET1-mediated RhoA activation facilitates lysophosphatidic acid-induced cell migration and invasion in gastric cancer. Br J Cancer 2008; 99: 1322-9.*
23. Sui L, Dong Y, Watanabe Y, Yamaguchi F, Sugimoto K, Tokuda M. *Clinical significance of Skp2 expression, alone and combined with Jab1 and p27 in epithelial ovarian tumors. Oncol Rep 2006; 15: 765-71.*
24. Osoegawa A, Yoshino I, Kometani T, Yamaguchi M, Kameyama T, Yohena T, Maehara Y. *Overexpression of Jun activation domain-binding protein 1 in nonsmall cell lung cancer and its significance in p27 expression and clinical features. Cancer 2006; 107: 154-61.*
25. Kouvaraki MA, Rassidakis GZ, Tian L, Kumar R, Kittas C, Claret FX. *Jun activation domain-binding protein 1 expression in breast cancer inversely correlates with the cell cycle inhibitor p27 (Kip1). Cancer Res 2003; 63: 2977-81.*
26. Sui L, Dong Y, Watanabe Y, Yamaguchi F, Sugimoto K, Tokuda M. *Clinical significance of Skp2 expression, alone and combined with Jab1 and p27 in epithelial ovarian tumors. Oncol Rep 2006; 15: 765-71.*
27. Hemler ME. *Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol 2005; 6: 801-11.*
28. Zoller M. *Tetraspanins: push and pull in suppressing and promoting metastasis. Nat Rev Cancer 2009; 9: 40-55.*
29. Shirane M, Harumiya Y, Ishida N, Hirai A, Miyamoto C, Hatakeyama S, Nakayama K, Kitagawa M. *Down-regulation of p27(Kip1) by two mechanisms, ubiquitin-mediated degradation and proteolytic processing. J Biol Chem 1999; 274: 13886-93.*

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