

Relation of overexpression of S phase kinase-associated protein 2 with reduced expression of p27 and PTEN in human gastric carcinoma

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Abstract

AIM: To investigate the significance of S phase kinase-associated protein 2 (Skp2) expression in human gastric carcinoma and the relation between expressions of Skp2, p27 and PTEN.

METHODS: Immunohistochemical analysis was performed on 138 gastric carcinoma specimens, their paired adjacent mucosa specimens, 102 paired lymphatic metastatic carcinoma tissue specimens, 30 dysplasia specimens, 30 intestinal metaplasia specimens, 10 chronic superficial gastritis specimens and 5 normal gastric mucosa specimens for Skp2 expression and on 138 gastric carcinoma specimens for p27 and PTEN expression.

RESULTS: Skp2 labeling frequency was significantly higher in intestinal metaplasia (12.68 ± 0.86) and adjacent mucosa (19.32 ± 1.22) than in normal gastric mucosa (0.53 ± 0.13) and chronic superficial gastritis (0.47 ± 0.19) ($P = 0.000$); in dysplasia (16.74 ± 0.82) than in intestinal metaplasia ($P = 0.000$); in gastric primary carcinoma (31.34 ± 2.17) than in dysplasia and adjacent mucosa ($P = 0.000$); in metastasis gastric carcinoma in lymph nodes (39.76 ± 2.00) than in primary gastric carcinoma ($P = 0.037$), respectively. Skp2 labeling frequency was positively associated with differentiation degree ($\rho = 0.315$, $P = 0.000$), vessel invasion ($\rho = 0.303$, $P = 0.000$) and lymph node metastasis ($\rho = 0.254$, $P = 0.000$) of gastric cancer. Expression of Skp2 was negatively associated with p27 ($\rho = -0.451$, $P = 0.000$) and PTEN ($\rho = -0.480$, $P = 0.000$) expression in gastric carcinoma. p27 expression was positively associated with PTEN expression in gastric carcinoma ($\rho = 0.642$, $P = 0.000$).

CONCLUSION: Skp2 overexpression may be involved

in carcinogenesis and progression of human gastric carcinoma *in vivo*, possibly via p27 proteolysis. PTEN may regulate the expression of p27 by negatively regulating Skp2 expression.

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Key words: Gastric carcinoma; Skp2; p27; PTEN

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INTRODUCTION

Dysregulation of the cell cycle is required for the formation of most malignant tumors. Progression of the cell cycle is controlled by interactions between cell cycle control proteins (cyclins) and their catalytically active cyclin-dependent kinase (CDKs). The activity of each cyclin-CDK complex is in turn regulated by several different mechanisms; the most important being negative regulation by CDK inhibitors^[1]. p27 is an inhibitor of cyclinE-CDK2 and cyclinA-CDK2, which drive cells from G₁ to S phase of the cell division cycle^[2,3]. Loss of p27 function therefore accelerates cell cycle progression and predisposes cells to malignant transformation, as is well illustrated by the observation of increased tumor incidence in hemizygous and homozygous p27-deleted mutant mice after carcinogen exposure^[4,5]. Many clinical studies also indicate that low levels of p27 are associated with high aggressiveness and poor prognosis in a large variety of malignant tumors^[2,3], including breast carcinoma^[6,7], colorectal carcinoma^[8], lung cancer^[9], prostate cancer^[10] and gastric carcinoma^[11].

The amount of p27 is mainly regulated by post-translational ubiquitin-proteasome-mediated proteolysis^[12]. Cell cycle-dependent degradation of p27 is dependent on phosphorylation at Thr¹⁸⁷ in late G₁ phase by CDK2 under positive regulation by cyclinE. Thr¹⁸⁷ phosphorylation is a necessary prerequisite for the sequential addition of ubiquitin molecules by an ubiquitin ligase complex, SCF^{skp2} composed of Skp1, Cull, Rbx1 and the F-box protein Skp2^[3]. Polyubiquitination of p27 then targets p27 for degradation in proteasome, thus removing the p27 cell

cycle “brake” and allowing cells to transition from G₁ to S phase^[13].

Some investigations have shown that Skp2 is a specific substrate-recognition subunit of SCF^{Skp2}, expression of Skp2 is required for the ubiquitination and subsequent degradation of p27 *in vitro*^[20-22], and Skp2 knock-out cells show high levels of p27^[16]. The level of p27 has also been reported to be inversely related to that of Skp2 in lymphoma^[24], oral squamous cell carcinoma^[18,19] and colorectal carcinoma^[20].

Recently, Mamillapalli *et al.*^[21] reported that PTEN, a tumor suppressor, regulates the ubiquitin-dependent degradation of p27 through SCF^{Skp2}. Yang *et al.*^[22] suggested that induction of Skp2 may be causally linked with decreased levels of p27 in prostate cancer and implicate PTEN in the regulation of Skp2 expression *in vivo*.

Recent studies have shown that Skp2 has oncogenic potential in breast epithelial cells and is overexpressed in a subset of breast carcinomas (ER- and Her-2 negative)^[23] and Skp2 can mediate transformation and is upregulated during oral epithelial carcinogenesis^[18]. Recently, a line of evidence also indicates a possible relationship between Skp2 expression and the malignancy of tumors. Skp2 expression has been shown to be greatly increased in malignantly transformed cell lines including oral squamous cell carcinoma^[18] and correlates directly with the grade of malignancy of lymphoma^[17] and oral squamous cell carcinoma^[18]. Kudo *et al.*^[19] reported that high Skp2 expression is also correlated with poor prognosis in oral squamous cell carcinoma. Thus, Skp2 may have a great significance in human carcinogenesis. However, few studies are available regarding the significance of Skp2 expression in human gastric carcinomas *in vivo* and there are no studies regarding the relationship between Skp2, p27 and PTEN expression. We therefore investigated the significance of Skp2 expression in human gastric carcinomas and the relationship between Skp2, p27 and PTEN expression *in vivo*.

MATERIALS AND METHODS

Patients and gastric specimens

One hundred and thirty-eight surgically resected gastric carcinoma specimens, paired adjacent mucosa specimens and paired 102 lymphatic gastric carcinoma tissue specimens metastatically selected from the Fourth Affiliated Hospital of Hebei Medical University were used in this study. All gastric carcinoma patients underwent total or subtotal gastrectomy and no patient received any treatment for cancer before surgery. All regional lymph nodes were removed. The patients comprised 111 males and 27 females with a mean age of 58.5 years and a median age of 60 years (from 36 to 78 years). After surgery, gastric specimens were fixed in 40 g/L neutral-buffered formaldehyde and embedded in paraffin. Additionally, 75 biopsy cases (5 cases of normal gastric mucosa, 10 cases of chronic superficial gastritis, 30 cases of intestinal

metaplasia and 30 cases of dysplasia) were included in this study as well. To avoid evaluator variability, all pathological diagnoses were done by two pathologists. Clinical stage was done according to the International Union Contraele Cancer criteria published in 1997.

Immunohistochemistry

A standard Non-Biotin HRP two-step immunohistochemical method (Zymed) was used. Four-micrometer-thick sections were deparaffinized and rehydrated. Endogenous peroxidase in sections was inactivated in 30 mL/L hydrogen peroxide (H₂O₂) in ethanol for 15 min at room temperature. The sections were washed thrice for 5 min with 0.01 mol/L phosphate-buffered saline (PBS) and then heated in a citrate buffer (0.01 mol/L, pH 6.4) for PTEN and p27 or in a EDTA buffer (1 mmol/L, pH 8.0) for Skp2 in an 800-W microwave oven for 12 min for antigen retrieval. The sections were incubated with mouse monoclonal antibody to Skp2 (1:50 dilution, Zymed), p27 (1:50 dilution, Santa Cruz) and PTEN (1:50 dilution, Zymed) overnight at 4 °C, washed thrice for 5 min with 0.01 mol/L PBS 3 and then incubated with common IgG (Fab fraction)-HRP complex for 30 min at room temperature. Immunoreactive products were visualized using 3,3'-diaminobenzidine/H₂O₂, and finally the sections were counterstained with hematoxylin. Some sections were incubated with PBS instead of primary antibody as negative control to verify the specificity of the immunoreactions. Vascular endothelial cells showed strong PTEN expression with a nuclear predominance and served as an internal positive control for PTEN in this study. The positive breast adenocarcinoma served as a positive control for Skp2 and p27.

Immunohistochemical quantitation

Immunostaining of nuclear Skp2, p27 and PTEN in each specimen was evaluated microscopically and recorded as the percentage of Skp2, p27, and PTEN-positive cells (labeling frequency), after at least 1 000 nuclei at the lesion site were calculated in at least five high-power fields (×400). All specimens were evaluated without any knowledge of the patients' clinical information.

Statistical analysis

The differences in Skp2 labeling frequencies among specimens of normal gastric mucosa, chronic superficial gastritis, intestinal metaplasia, dysplasia, gastric adenocarcinoma, adjacent mucosa and lymphatic metastatic gastric carcinoma were compared by Mann-Whitney *U* test. The relation between Skp2 expression and the clinical and pathological variables was evaluated using the Spearman correlation coefficient. The Spearman's correlation coefficient testing was also used to determine the relation between Skp2, PTEN and p27 as well as between PTEN and p27. *P* < 0.05 was considered statistically significant. All analyses were performed using SPSS 11.0 statistical software.

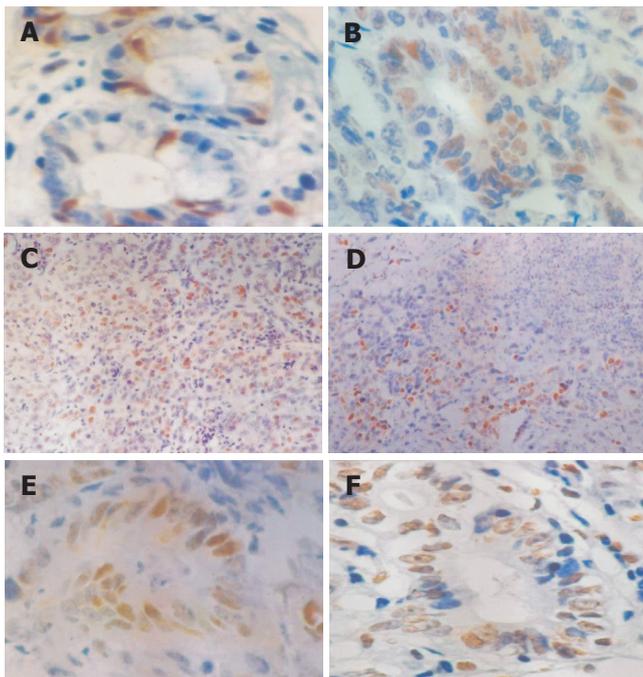


Figure 1 Skp2, p27 and PTEN expression in different gastric tissues (DAB and hematoxylin stain). **A:** Positive Skp2 in nuclei of intestinal metaplasia and dysplasia cells (original magnification $\times 400$); **B:** Positive Skp2 in nuclei of well-differentiated gastric carcinoma cells (original magnification $\times 400$); **C:** Positive Skp2 in nuclei of poorly differentiated gastric carcinoma cells (original magnification $\times 100$); **D:** Positive Skp2 in lymphatic metastatic gastric carcinoma cells (original magnification $\times 100$); **E:** Positive p27 in nuclei of well-differentiated gastric carcinoma cells (original magnification $\times 400$); **F:** Positive PTEN in nuclei of well-differentiated gastric carcinoma cells (original magnification $\times 400$).

RESULTS

Expression of Skp2 in human gastric specimen

The Skp2 immunoreactivity was predominantly localized in the nuclei of gastric cells (Figure 1). In 5% of the cancer specimens examined, a weak or moderate cytoplasmic immunoreactivity could be seen in addition to the predominant nuclear reactivity in cancer cells. Skp2 labeling frequency in normal gastric mucosa, intestinal metaplasia, dysplasia, primary gastric carcinoma and lymphatic metastatic gastric carcinoma was significantly higher than in normal gastric mucosa and chronic superficial gastritis ($P = 0.000$); it was in dysplasia than in intestinal metaplasia ($P = 0.000$); in primary gastric carcinoma than in dysplasia ($P = 0.000$); in lymphatic metastatic gastric carcinoma than in primary gastric carcinoma ($P = 0.037$, Table 1).

Relation between Skp2 labeling frequency and clinicopathological features in gastric carcinoma

Relation between Skp2 labeling frequency and clinicopathological variables including age, gender, histological differentiation, depth of invasion, vessel invasion, lymphatic metastasis, distant metastasis as well as clinical stage is summarized in Table 2. Skp2 labeling frequency was negatively associated with age, gender, depth of invasion, distant metastasis as well as clinical stage. A significant correlation was found between the

Table 1 Skp2 protein expression in human gastric tissues

Tissue	Specimens (n)	Skp2 labeling frequency (%)	
		Median	Mean \pm SE
Normal gastric mucosa	5	0.00	0.47 \pm 0.19
Chronic superficial gastritis	10	0.00	0.53 \pm 0.13
Intestinal metaplasia	30	12.00	12.68 \pm 0.86
Dysplasia	30	19.00	16.74 \pm 0.82
Primary gastric carcinoma	138	29.00	31.34 \pm 2.17
Adjacent mucosa	138	16.00 ⁶	19.32 \pm 1.22
Metastasis tumor tissue in lymph node	102	39.50 ⁷	39.76 \pm 2.00

^{1,2,3,4}Frequency was significantly higher than in normal gastric tissue and chronic superficial gastritis ($P = 0.000$, all the same; Mann-Whitney U test). ²Frequency was significantly higher than in intestinal metaplasia ($P = 0.000$; Mann-Whitney U test). ³Frequency was significantly higher than in intestinal metaplasia and dysplasia, ($P = 0.000$, all the same; Mann-Whitney U test). ⁵Frequency was significantly higher than in primary tumors ($P = 0.037$; Mann-Whitney U test).

Table 2 Skp2 protein expression in 138 human gastric cancers

Clinicopathological feature	Specimens (n)	Skp2 labeling frequency (%)	
		Mean \pm SE	rho P
Patients	138	36.32 \pm 1.22	
Age (yr)	138	58.50	0.190 0.833
<60	66	34.25 \pm 3.58	
≥ 60	72	34.41 \pm 2.63	
Gender			0.119 0.179
Females	27	24.96 \pm 5.21	
Males	111	32.90 \pm 2.37	
Differentiation grade			0.315 0.000
Well/Moderate	63	22.41 \pm 2.59	
Poor	75	38.85 \pm 3.10	
Depth of invasion			0.001 0.986
Serosal	24	25.84 \pm 2.45	
Outside soft tissue	114	29.25 \pm 4.87	
Vessel invasion			0.303 0.000
Absent	120	27.89 \pm 2.11	
Present	18	54.39 \pm 6.84	
Lymph node metastasis			0.254 0.000
Absent	36	27.94 \pm 3.38	
Present	102	37.14 \pm 2.69	
Distant metastasis			0.091 0.307
Absent	26	22.77 \pm 6.92	
Present	12	31.79 \pm 2.38	
Clinical stage			0.069 0.440
0	0		
Ia	0		
Ib	3	22.78 \pm 6.92	
II	36	29.81 \pm 3.14	
IIIa	12	44.67 \pm 6.78	
IIIb	66	34.50 \pm 3.55	
IV	9	53.00 \pm 0.57	

^{1,2,3}Skp2 labeling frequency was positively correlated with differentiated degree (rho = 0.315, $P = 0.000$), vessel invasion (rho = 0.303, $P = 0.000$), and lymph node metastasis (rho = 0.254, $P = 0.000$) in gastric carcinoma, respectively. ⁴Because clinical staging could not be performed due to the lack of accurate records about lymph node in operation, data of 12 cases were cancelled in statistical analysis.

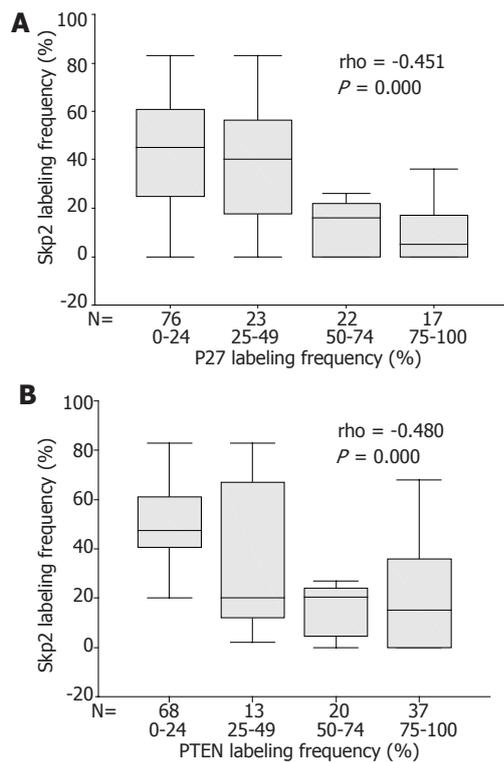


Figure 2 Correlation of Skp2 with p27 (A) and PTEN (B) protein levels. Each box and the associated bars represent the values of middle 50% and the range of the data respectively. The dark line within a box denotes the median.

Skp2 labeling frequency and differentiation degree ($\rho = 0.315$, $P = 0.000$), vessel invasion ($\rho = 0.303$, $P = 0.000$) and lymphatic metastasis ($\rho = 0.254$, $P = 0.000$). Poorly differentiated gastric carcinoma, gastric carcinoma with vessel invasion and gastric carcinoma with lymphatic metastasis tended to have a higher Skp2 labeling frequency.

Relation between Skp2, p27 and PTEN expression in gastric carcinoma

Given the biochemical link between Skp2 and p27, we asked whether there was a correlation between Skp2 and p27 levels in gastric carcinoma using the same set of gastric carcinoma specimens used for the Skp2 analysis. A statistically significant inverse relation between p27 and Skp2 labeling frequency was evident ($\rho = -0.451$, $P = 0.000$; Figure 2A). *In vitro* study linking loss of PTEN with increased Skp2 levels led us^[29] to also compare the expression of PTEN and Skp2 using the same set of gastric carcinoma specimens. Interestingly, we found that increased Skp2 expression was significantly correlated with loss and decrease of PTEN expression in gastric carcinoma ($\rho = -0.480$, $P = 0.000$; Figure 2B). Additionally, a positive relation between p27 and PTEN expression was observed in gastric carcinoma ($\rho = 0.642$, $P = 0.000$; Figure 3).

DISCUSSION

Protein degradation by the ubiquitin–proteasome pathway

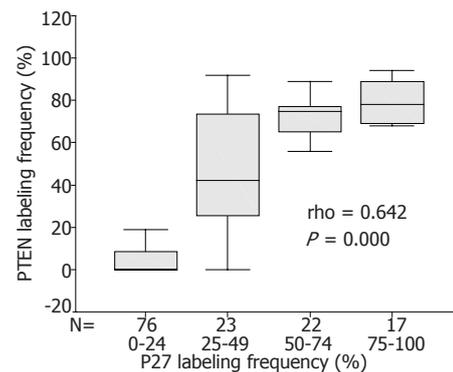


Figure 3 Correlation of p27 and PTEN expression in human gastric carcinoma. Each box and the associated bars represent the values of middle 50% and the range of the data respectively. The dark line within a box denotes the median.

plays a fundamental role in the regulation of eukaryotic cell cycle. Proteolysis of G₁ regulatory proteins is mediated by SCF ubiquitin ligase complex (SCF^{Skps}) composed of four major subunits, Skp1, Cul1, Rbx1/Roc1 and one of the F-box proteins (Fbps)^[24]. There are 11 Fbps in budding yeast, 22 in *Drosophila*^[25] and 38 in human beings^[26,27]. Skp2 is a Fbp first identified together with Skp1 as an interactor of the cyclinA-Cdk2 complex which drives cells from G₁ to S phase, hence it is named as S phase kinase-associated protein 2 (Skp2)^[24].

In this study, we found that Skp2 immunoreactivity was rare in normal gastric mucosa and chronic superficial gastritis, but Skp2 labeling frequency was significantly increased during the course of intestinal metaplasia, dysplasia and primary gastric carcinoma. From pathological point of view, development of gastric adenocarcinoma involves progression through a well-defined series of histological steps initiated by the change of normal mucosa to chronic superficial gastritis, followed by the appearance of atrophic gastritis and intestinal metaplasia, then dysplasia and finally adenocarcinoma^[28]. These results suggest that Skp2 may have oncogenic potential in gastric carcinoma and is involved in gastric carcinogenesis. It may become a new biomarker in gastric carcinogenesis. Additionally, we found that Skp2 labeling frequency in adjacent mucosa of gastric carcinoma was significantly higher than that in normal gastric mucosa, suggesting that some adjacent mucosae of gastric carcinoma have normal histological structure, but the expression of some proteins is changed.

In this study, we found that Skp2 labeling frequency was positively correlated with differentiation degree, lymphatic metastasis and vessel invasion of gastric carcinoma. Poorly differentiated gastric carcinoma and gastric carcinoma with lymphatic metastasis and vessel invasion tended to have a higher Skp2 labeling frequency. Skp2 labeling frequency was higher in lymphatic metastatic gastric carcinoma than in primary gastric carcinoma. The overexpression of Skp2 could modulate the malignant phenotype of gastric carcinoma cells, suggesting that overexpression of Skp2 may be associated with metastasis

potential of gastric carcinoma cells. Further studies need to be done to clarify their mechanism.

In the present study, we also found that Skp2 expression was inversely correlated with the expression of p27, which is consistent with the results reported in other studies^[18,19,21]. These results suggest that Skp2 overexpression is associated with p27 protein degradation in cancer tissue, which may be a reason why Skp2 overexpression is involved in carcinogenesis and progression of cancer. Furthermore, Skp2 expression was also inversely correlated with PTEN expression and p27 expression was positively related with PTEN expression in gastric carcinoma. PTEN tumor suppressor acts as a phosphatase of phosphatidylinositol-3,4,5-trisphosphate (PIP3) and negatively controls the G₁/S cell cycle transition and regulates the levels of p27^[29]. Mamillapalli *et al*^[21] showed that PTEN deficiency in mouse embryonic stem cells decreases p27 level while increases Skp2 level. Conversely, in human glioblastoma cells, ectopic PTEN expression leads to p27 accumulation accompanied with a reduction of Skp2 and ectopic expression of Skp2 alone is sufficient to reverse PTEN-induced p27 accumulation, restore the kinase activity of cyclin E/CDK2 and partially overcome the PTEN-induced G₁ cell cycle arrest. Recombinant SCF^{Skp2} complex or Skp2 protein alone can rescue the defect in p27 ubiquitination in extracts from cells treated with a PI 3-kinase inhibitor. Yang *et al*^[30] showed that loss of expression or reduced expression of PTEN protein contributes to carcinogenesis and progression of gastric carcinoma. Myung *et al*^[31] reported that reduced expression of cyclin-dependent kinase inhibitor p27 is associated with advanced stage and invasiveness of gastric carcinoma. Additionally, Yang *et al*^[22] also found that elevated Skp2 protein expression in human prostate cancer is associated with loss of p27 and PTEN expression. Thus, all these findings suggest that PTEN functions as a negative regulator of the Skp2 pathway that is normally used to control S-phase entry through the regulation of p27 in gastric carcinoma and the effects of Skp2, p27 and PTEN together play an important role in carcinogenesis and progression of gastric carcinoma. These results also suggest that Skp2 functions as a critical component in the PTEN/PI3-kinase pathway for the regulation of p27.

In conclusion, alterations in the levels of proteins controlled by the ubiquitin–proteasome pathway during transformation are likely to be common. Further studies that define how such posttranscriptional control pathways are altered during transformation may provide additional biomarkers or facilitate the identification of novel therapeutic targets. The finding that Skp2 is induced in a number of different cancers suggests that drugs directed at this molecule may provide a more selective target for therapeutic development.

REFERENCES

- Sherr CJ. Cancer cell cycles. *Science* 1996; **274**: 1672-1677
- Philipp-Staheli J, Payne SR, Kemp CJ. p27(Kip1): regulation and function of a haploinsufficient tumor suppressor and its misregulation in cancer. *Exp Cell Res* 2001; **264**: 148-168
- Slingerland J, Pagano M. Regulation of the cdk inhibitor p27 and its deregulation in cancer. *J Cell Physiol* 2000; **183**: 10-17
- Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ. The murine gene p27Kip1 is haplo-insufficient for tumour suppression. *Nature* 1998; **396**: 177-180
- Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Hori I, Loh DY, Nakayama K. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* 1996; **85**: 707-720
- Tan P, Cady B, Wanner M, Worland P, Cukor B, Magi-Galluzzi C, Lavin P, Draetta G, Pagano M, Loda M. The cell cycle inhibitor p27 is an independent prognostic marker in small (T1a,b) invasive breast carcinomas. *Cancer Res* 1997; **57**: 1259-1263
- Catzavelos C, Bhattacharya N, Ung YC, Wilson JA, Roncari L, Sandhu C, Shaw P, Yeger H, Morava-Protzner I, Kapusta L, Franssen E, Pritchard KL, Slingerland JM. Decreased levels of the cell-cycle inhibitor p27Kip1 protein: prognostic implications in primary breast cancer. *Nat Med* 1997; **3**: 227-230
- Loda M, Cukor B, Tam SW, Lavin P, Fiorentino M, Draetta GF, Jessup JM, Pagano M. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. *Nat Med* 1997; **3**: 231-234
- Esposito V, Baldi A, De Luca A, Groger AM, Loda M, Giordano GG, Caputi M, Baldi F, Pagano M, Giordano A. Prognostic role of the cyclin-dependent kinase inhibitor p27 in non-small cell lung cancer. *Cancer Res* 1997; **57**: 3381-3385
- Tsihlias J, Kapusta LR, DeBoer G, Morava-Protzner I, Zbieranowski I, Bhattacharya N, Catzavelos GC, Klotz LH, Slingerland JM. Loss of cyclin-dependent kinase inhibitor p27Kip1 is a novel prognostic factor in localized human prostate adenocarcinoma. *Cancer Res* 1998; **58**: 542-548
- Ohtani M, Isozaki H, Fujii K, Nomura E, Niki M, Mabuchi H, Nishiguchi K, Toyoda M, Ishibashi T, Tanigawa N. Impact of the expression of cyclin-dependent kinase inhibitor p27Kip1 and apoptosis in tumor cells on the overall survival of patients with non-early stage gastric carcinoma. *Cancer* 1999; **85**: 1711-1718
- Pagano M, Tam SW, Theodoras AM, Beer-Romero P, Del Sal G, Chau V, Yew PR, Draetta GF, Rolfe M. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science* 1995; **269**: 682-685
- Eguchi H, Herschenhou N, Kuzushita N, Moss SF. Helicobacter pylori increases proteasome-mediated degradation of p27(kip1) in gastric epithelial cells. *Cancer Res* 2003; **63**: 4739-4746
- Carrano AC, Eytan E, Hershko A, Pagano M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat Cell Biol* 1999; **1**: 193-199
- Tsvetkov LM, Yeh KH, Lee SJ, Sun H, Zhang H. p27(Kip1) ubiquitination and degradation is regulated by the SCF(Skp2) complex through phosphorylated Thr187 in p27. *Curr Biol* 1999; **9**: 661-664
- Nakayama K, Nagahama H, Minamishima YA, Matsumoto M, Nakamichi I, Kitagawa K, Shirane M, Tsunematsu R, Tsukiyama T, Ishida N, Kitagawa M, Nakayama K, Hatakeyama S. Targeted disruption of Skp2 results in accumulation of cyclin E and p27(Kip1), polyploidy and centrosome overduplication. *EMBO J* 2000; **19**: 2069-2081
- Latres E, Chiarle R, Schulman BA, Pavletich NP, Pellicer A, Inghirami G, Pagano M. Role of the F-box protein Skp2 in lymphomagenesis. *Proc Natl Acad Sci USA* 2001; **98**: 2515-2520
- Gstaiger M, Jordan R, Lim M, Catzavelos C, Mestan J, Slingerland J, Krek W. Skp2 is oncogenic and overexpressed in human cancers. *Proc Natl Acad Sci USA* 2001; **98**: 5043-5048
- Kudo Y, Kitajima S, Sato S, Miyauchi M, Ogawa I, Takata T. High expression of S-phase kinase-interacting protein 2, human F-box protein, correlates with poor prognosis in oral squamous cell carcinomas. *Cancer Res* 2001; **61**: 7044-7047

- 20 **Hershko D**, Bornstein G, Ben-Izhak O, Carrano A, Pagano M, Krausz MM, Hershko A. Inverse relation between levels of p27(Kip1) and of its ubiquitin ligase subunit Skp2 in colorectal carcinomas. *Cancer* 2001; **91**: 1745-1751
- 21 **Mamillapalli R**, Gavrilova N, Mihaylova VT, Tsvetkov LM, Wu H, Zhang H, Sun H. PTEN regulates the ubiquitin-dependent degradation of the CDK inhibitor p27(KIP1) through the ubiquitin E3 ligase SCF(SKP2). *Curr Biol* 2001; **11**: 263-267
- 22 **Yang G**, Ayala G, De Marzo A, Tian W, Frolov A, Wheeler TM, Thompson TC, Harper JW. Elevated Skp2 protein expression in human prostate cancer: association with loss of the cyclin-dependent kinase inhibitor p27 and PTEN and with reduced recurrence-free survival. *Clin Cancer Res* 2002; **8**: 3419-3426
- 23 **Signoretti S**, Di Marcotullio L, Richardson A, Ramaswamy S, Isaac B, Rue M, Monti F, Loda M, Pagano M. Oncogenic role of the ubiquitin ligase subunit Skp2 in human breast cancer. *J Clin Invest* 2002; **110**: 633-641
- 24 **Carrano AC**, Pagano M. Role of the F-box protein Skp2 in adhesion-dependent cell cycle progression. *J Cell Biol* 2001; **153**: 1381-1390
- 25 **Rubin GM**, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, Cherry JM, Henikoff S, Skupski MP, Misra S, Ashburner M, Birney E, Boguski MS, Brody T, Brokstein P, Celniker SE, Chervitz SA, Coates D, Cravchik A, Gabrielian A, Galle RF, Gelbart WM, George RA, Goldstein LS, Gong F, Guan P, Harris NL, Hay BA, Hoskins RA, Li J, Li Z, Hynes RO, Jones SJ, Kuehl PM, Lemaitre B, Littleton JT, Morrison DK, Mungall C, O'Farrell PH, Pickeral OK, Shue C, Vossell LB, Zhang J, Zhao Q, Zheng XH, Lewis S. Comparative genomics of the eukaryotes. *Science* 2000; **287**: 2204-2215
- 26 **Cenciarelli C**, Chiaur DS, Guardavaccaro D, Parks W, Vidal M, Pagano M. Identification of a family of human F-box proteins. *Curr Biol* 1999; **9**: 1177-1179
- 27 **Winston JT**, Koeppe DM, Zhu C, Elledge SJ, Harper JW. A family of mammalian F-box proteins. *Curr Biol* 1999; **9**: 1180-1182
- 28 **Konturek PC**, Kania J, Konturek JW, Nikiforuk A, Konturek SJ, Hahn EG. H.pylori infection, atrophic gastritis, cytokines, gastrin, COX-2, PPAR gamma and impaired apoptosis in gastric carcinogenesis. *Med Sci Monit* 2003; **9**: SR53-SR66
- 29 **Gottschalk AR**, Basila D, Wong M, Dean NM, Brandts CH, Stokoe D, Haas-Kogan DA. p27Kip1 is required for PTEN-induced G1 growth arrest. *Cancer Res* 2001; **61**: 2105-2111
- 30 **Yang L**, Kuang LG, Zheng HC, Li JY, Wu DY, Zhang SM, Xin Y. PTEN encoding product: a marker for tumorigenesis and progression of gastric carcinoma. *World J Gastroenterol* 2003; **9**: 35-39
- 31 **Myung N**, Kim MR, Chung IP, Kim H, Jang JJ. Loss of p16 and p27 is associated with progression of human gastric cancer. *Cancer Lett* 2000; **153**: 129-136

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