

# Jab1 has negative effects on p53-mediated genotoxic stresses

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**In this study, we use promoter analysis to show that interaction between Jab1 and p53 induces suppression of p53 activation in U2OS and H1299 cells. Interaction between p53 and Jab1 was further confirmed by immunoprecipitation and immunofluorescent analyses. In particular, Jab1 was able to induce nuclear export of p53 as previously reported. When Jab1 was overexpressed in U2OS cells followed by etoposide or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), cell death induced by such stresses was protected against. On the contrary, when the level of Jab1 was suppressed in U2OS cells, cytotoxicity imposed by etoposide and H<sub>2</sub>O<sub>2</sub> was dramatically increased, suggesting a cell protective role for Jab1. These results indicate that Jab1 is a negative regulator of p53 and a plausible oncogene. [BMB reports 2009; 42(5): 299-303]**

## INTRODUCTION

p53 is a transcription factor known as the genome gatekeeper. It functions as a tumor suppressor by inducing cell cycle arrest or apoptosis (1, 2). Due to its potent cytotoxicity and its ability to maintain genomic integrity, its roles in cancer prevention have been deemed essential (3). Under normal conditions, the level of p53 is kept low due to its target protein, Hdm2, which is an E3 ligase that induces poly-ubiquitination of p53 (2, 4, 5). Hdm2 is also involved in the nuclear export of p53 by inducing mono-ubiquitination of p53 (6, 7). It is assumed that p53 when localized in the cytoplasm is prevented from functioning as a transcriptional activator, which induces cell cycle arrest or apoptosis. Therefore, there seems to be a delicate balance of controlling p53 activities in the cytoplasm.

Jab1 (also known as CSN5) as the fifth member of the CSN complex was first identified as a c-Jun and JunD transcriptional activator (8), and is involved in a variety of regulatory pathways. For example, Jab1 binds to cyclin E, p27<sup>kip1</sup>, SCF ubiquitin ligase and Smad family proteins (9-13). Interestingly, Jab1 as a component of the CSN complex binds to p53 and induces phosphorylation, which facilitates p53 degradation in a proteasome-

pendent pathway (14). Jab1 alone induces nuclear export of p53 as well as its degradation in a proteasome-dependent manner (15, 16). In these studies, it was shown that nuclear export of p53 is accompanied by a degradation mechanism that suggests the existence of a new cytoplasmic E3 ligase. The physiological evidence that Jab1 regulates p53 levels was shown in Jab1- knock-out mice, which exhibit higher p53 and p27 levels (17).

Presently, we demonstrate the ability of Jab1 to protect cells from p53-induced cytotoxicity. The protective effects of Jab1 were confirmed by examining the effects of overexpressing and suppressing Jab1 in cells.

## RESULTS AND DISCUSSION

### Jab1 suppresses p53 activity under stressed conditions

Jab1 normally induces nuclear export of p53 followed by its degradation (15). However, these activities do not occur under stress. Under stressed conditions, p53 is prevented from interacting with Hdm2, thereby inhibiting Hdm2-mediated p53 mono-ubiquitination and the subsequent export of p53. Since Jab1 can bind to p53, we tested whether expression of Jab1 is able to prevent p53 transcriptional activity. H1299 cells were transfected with a vector containing the p53 responsive promoter, Bax-luc (Fig. 1A). Transfection of p53 followed by etoposide treatment produced a 5-fold increase in p53 activity. Upon coexpression with Jab1, p53 activity was reduced by up to 50%, thus indicating that Jab1 was able to suppress p53 activity. These results were obtained from H<sub>2</sub>O<sub>2</sub> stress. We next transfected Bax-luc into U2OS cells treated simultaneously with etoposide or H<sub>2</sub>O<sub>2</sub> (Fig. 1B). Treatment of etoposide or H<sub>2</sub>O<sub>2</sub> induced activation of p53. However, when Jab1 was incorporated to the system, p53 activity was reduced under both stresses. This suggests Jab1 is able to suppress p53 activity. Overall the data indicate that Jab1 is able to suppress p53 activity under stressed conditions.

### Jab1 binds to p53 and induces nuclear export

To confirm the ability of Jab1 to bind to p53, immunoprecipitation analysis was performed. p53 and Jab1 were able to interact to each other (Fig. 2A), as has been shown before (15). To further examine the interaction between Jab1 and p53, the ability of Jab1 to translocate p53 was investigated. As shown in Fig. 2B, around 80% of p53 was localized to the nucleus. On the contrary, Jab1 induced the cytoplasmic localization of p53 by up to 70% (Fig. 2B). These data suggest that Jab1 is able to bind to p53 while simultaneously promoting nuclear export of p53.

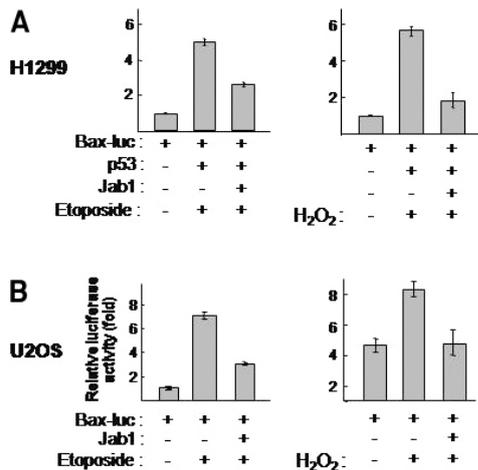
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### Jab1 has negatively regulates p53-mediated cell death

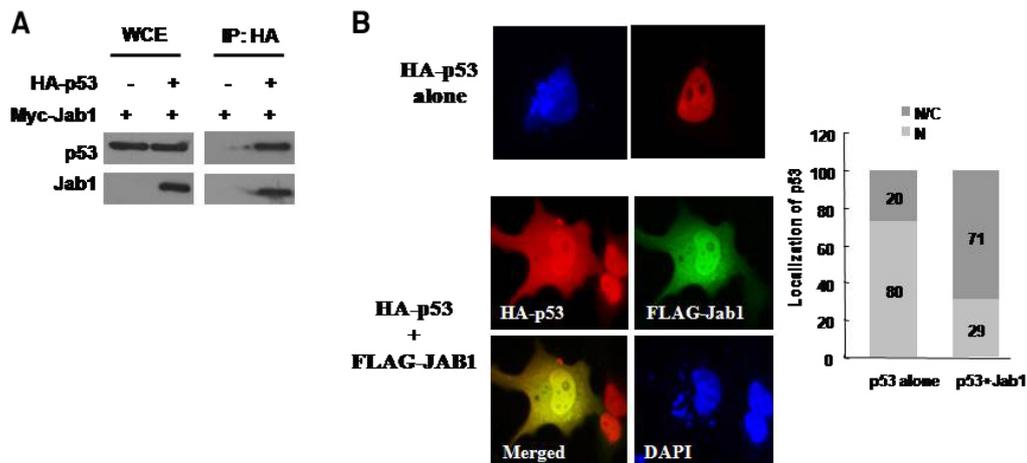
Jab1-mediated export of p53 is inhibited by treatment with various stress agents, such as etoposide or H<sub>2</sub>O<sub>2</sub> (15). However, the ability of Jab1 to suppress p53 activity remains intact (Figs. 1A and



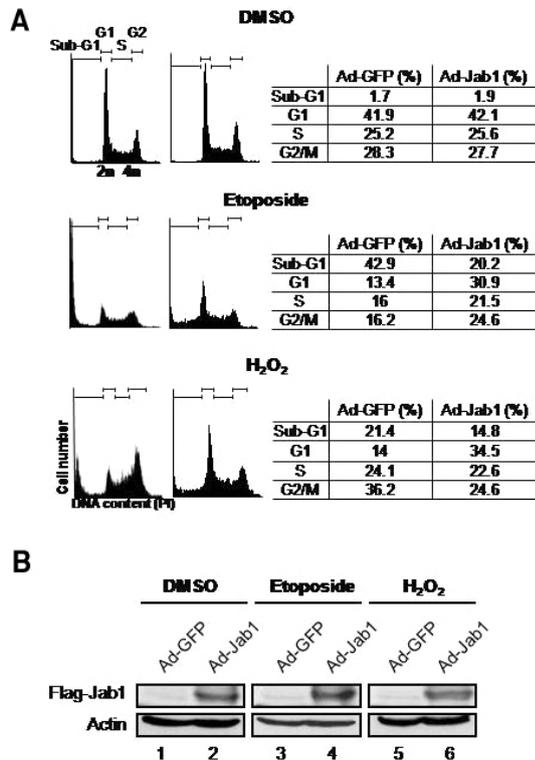
**Fig. 1.** Jab1 suppresses p53 activity. (A) H1299 cells were cotransfected with the p53 regulatory gene promoter, Bax-luc, together with pcDNA or combinations of plasmids expressing HA-p53 or FLAG-Jab1 in the presence of etoposide or H<sub>2</sub>O<sub>2</sub>. (B) U2OS cells were cotransfected with Bax-luc together with pcDNA or plasmids expressing FLAG-Jab1 in the presence of etoposide or H<sub>2</sub>O<sub>2</sub>. The luciferase activities were measured 48 h after transfection. Three independent experiments were performed and the standard deviation is indicated by error bars.

1B). These observations suggest that Jab1 might exert protective effects against various stress agents that induce p53 activity. To verify this, we used adenovirus to overexpress Jab1 in U2OS cells, monitoring their cell cycle under etoposide and H<sub>2</sub>O<sub>2</sub> treatment (Figs. 3A and 3B). Etoposide or H<sub>2</sub>O<sub>2</sub> treatment induced sub-G1 fractions by up to 42.9% and 21.5%, respectively (Fig. 3A). When Jab1 was overexpressed, the sub-G1 fractions of U2OS cells treated with etoposide or H<sub>2</sub>O<sub>2</sub> were reduced by 20.2% and 14.8%, respectively. These observations indicate that Jab1 was able to provide cell protection against p53-inducing agents possibly by directly suppressing p53 activity. Next, we examined the effect of Jab1 depletion on U2OS cells treated with etoposide or H<sub>2</sub>O<sub>2</sub> (Fig. 4). Jab1 small interfering RNA (siRNA) reduced endogenous levels of Jab1 up to 90% under normal or stress conditions (Fig. 4B). Ablation of Jab1 did not have any effect on the U2OS cell cycle under normal conditions (Fig. 4A, upper panel). Etoposide or H<sub>2</sub>O<sub>2</sub> treatment resulted in sub-G1 fractions being increased by up to 43.2% and 26.7%, respectively (Fig. 4A). When Jab1 was ablated, significant increases of 87.2% and 62.1% were observed in sub-G1 fractions upon etoposide or H<sub>2</sub>O<sub>2</sub> treatment, respectively. These results indicate that endogenous Jab1 might have a regulatory effect on endogenous p53 activated by stress reagents, specifically, that Jab1 is a negative regulator of p53. Overexpression of Jab1 endows cells with protective effects that decrease cell death. On the contrary, ablation of Jab1 significantly increased cell death induced by etoposide or H<sub>2</sub>O<sub>2</sub>.

The present study suggests that Jab1 might be an oncogene that suppresses p53 activity. Jab1 is overexpressed in the brain, ovary, pituitary, breast, lung and pancreas (18-25). These clinical reports, along with the present biochemical data, indicate that Jab1 might mediate suppressive effects on p53 activation. The mecha-



**Fig. 2.** Interaction of Jab1 with p53. (A) Plasmids expressing HA-p53 and 6xMyc-Jab1 were cotransfected into 293T cells. Cell lysates were immunoprecipitated using anti-HA antibody. Whole cell extracts (WCE) and immunoprecipitates (IP) were detected using anti-HA and anti-Myc antibodies. (B) H1299 cells were transfected with plasmids expressing HA-p53 and/or FLAG-Jab1. The cells were immunostained using anti-FLAG and Alexa Fluor 488 anti-mouse antibodies, or with polyclonal anti-HA and Alexa 594 anti-rabbit antibodies. The cells were counterstained with DAPI to visualize the nuclei. Cells expressing HA-p53 were counted according to their localization as follows: nucleus (N) or cytoplasm and nucleus (N/C). A total of 200 cells were counted for each experiment.



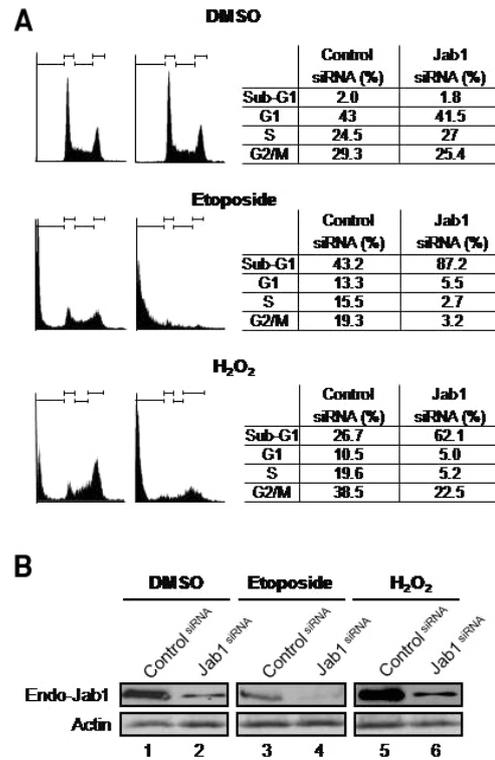
**Fig. 3.** Ectopic expression of Jab1 prevents genotoxic stress-induced cell death. (A) U2OS cells infected with adenovirus expressing either GFP or Flag-Jab1 were treated with dimethylsulfoxide (top panel), 50  $\mu$ M etoposide for 30 h (mid panel), or 1 mM H<sub>2</sub>O<sub>2</sub> for 6 h (bottom panel). The cells were harvested, fixed and stained using propidium iodide, followed by FACS analysis. (B) WCE from adenoviral-infected U2OS cells were analyzed by Western blotting using anti-Jab1 and anti-Actin antibodies.

nism through which Jab1 negatively regulates p53 could be wide-ranging. Firstly, Jab1 can induce phosphorylation of the p53 residues 149, 150 and 155 as a component of the CSN complex (14). It can also induce nuclear export of p53 concomitant with its degradation (15). Finally, it interacts with p53 and suppresses its transcriptional activity, which prevents cell death. These observations implicate Jab1 as a possible drug target for the therapeutic treatment of cancer.

## MATERIALS AND METHODS

### Plasmids and adenoviral vectors

pCDNA3-HA-p53, pCMV-Flag-Jab1 and pCS3-MT-BX (6xMyc)-Jab1 were used as previously described (15). Recombinant adenovirus (Ad/Jab1) was prepared using the AdEasy XL system according to manufacturer's instructions (Stratagene, Mountain View, CA). Viral stocks were produced by transfection of recombinant adenovirus genomes into 293 cells. Recombinant adenovirus (Ad/GFP) was a gift from J.S. Yang. All constructs were



**Fig. 4.** Depletion of Jab1 by siRNA sensitizes cells to cell death induced by etoposide or H<sub>2</sub>O<sub>2</sub>. (A) U2OS cells pretreated with control or Jab1 siRNA were treated with dimethylsulfoxide (top panel), 50  $\mu$ M etoposide for 30 h (mid panel), or 1 mM H<sub>2</sub>O<sub>2</sub> for 6 h (bottom panel). FACS analysis was performed as described in Fig. 3A. (B) WCE from the siRNA treatment were analyzed by Western blotting using anti-Jab1 and anti-Actin antibodies.

confirmed by enzyme digestion and DNA sequence analysis.

### Cell culture and DNA transfection

Human cancer cell lines H1299 (p53 null Lung carcinoma) and U2OS cells (Osteosarcoma) were maintained in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA). Transient transfections were performed using Lipofectamine plus reagent (Invitrogen) or Effectene (Qiagen, Valencia, CA), according to the manufacturer's recommendations.

### Antibodies and chemicals

Jab1 (FL-334), p53 (FL-393), p53 (DO-1), HA mouse (F-7), HA rabbit (Y-11) and GFP (FL) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Flag M2 antibodies were purchased from Sigma-Aldrich (St. Louis, MO).

### Immunofluorescence staining

Cells were plated in 6-well plates with coverslips. Transfections were performed using Lipofectamine reagent. After 24 h, cells

were fixed with 4% paraformaldehyde solution for 15 min at room temperature and then washed with phosphate buffered saline (PBS; Invitrogen) and permeabilized with 0.5% Triton X-100 in PBS for 15 min. The cells were blocked with 5% bovine serum albumin (Santa Cruz Biotechnology) in PBS for 30 min and incubated overnight with specific primary antibody at room temperature. The samples were then incubated with Alexa Fluor 488 anti-mouse or Alexa 594 anti-rabbit antibodies (each diluted 1:400) for 1 h at room temperature. The cells were stained with 4, 6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) for 7 min. The slides were analyzed using a LSM510 confocal microscope or 5203 Axiophot II immunofluorescent microscope (Carl Zeiss, Oberkochen, Germany).

### Biochemical analysis

Immunoprecipitation and Western blot analysis were performed as previously described (15). Luciferase activities were measured using a dual-luciferase assay kit (Promega, Madison, WI) according to the manufacturer's instructions. The Renilla luciferase reporter plasmid, pRL-CMV, was used as an internal control. The levels of endogenous Jab1 were reduced by Jab1 siRNA (5'-GCU CAGAGUAUCGAUGAAA-3') with 3'dTdT overhangs, as synthesized by QIAGEN (Valencia, CA). Control siRNA was a mixture of scrambled siRNA oligonucleotides (QIAGEN). Cells were transfected with 200 nM of siRNA using oligofectamine according to manufacturer's instructions (Invitrogen). Cells were subjected to immunoblotting or immunofluorescence after 72 h of transfection. For examination of cell death, H1299 and U2OS cells were seeded, transfected and treated with two genotoxic stresses, etoposide (25  $\mu$ M) and H<sub>2</sub>O<sub>2</sub> (1 mM), for 24 h. For fluorescence-activated cell sorting (FACS) analysis, U2OS cells were either infected with adenoviral-green fluorescent protein (GFP) or adenoviral-GFP/Jab1, or were transfected with control or Jab1 siRNA. The cells were then treated with etoposide or H<sub>2</sub>O<sub>2</sub> and then harvested and fixed with 70% ethanol. The fixed cells were stained with propidium iodide (50  $\mu$ g/ml) containing RNase (1  $\mu$ g/ml) and then detected by flow cytometry analysis (Becton-Dickinson, Franklin Lakes, NJ). The data were analyzed using CellQuest Pro software (Becton-Dickinson).

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### REFERENCES

1. Lane, D. P. (1992) Cancer. p53, guardian of the genome. *Nature* **358**, 15-16.
2. Levine, A. J. (1997) p53, the cellular gatekeeper for growth and division. *Cell* **88**, 323-331.
3. Vousden, K. H. and Lu, X. (2002) Live or let die: the cell's response to p53. *Nat. Rev. Cancer* **2**, 594-604.
4. O'Brate, A. and Giannakakou, P. (2003) The importance of p53 location: nuclear or cytoplasmic zip code? *Drug Resist. Updat.* **6**, 313-322.
5. Yang, Y., Li, C. C. and Weissman, A. M. (2004) Regulating the p53 system through ubiquitination. *Oncogene* **23**, 2096-2106.
6. Li, M., Brooks, C. L., Wu-Baer, F., Chen, D., Baer, R. and Gu, W. (2003) Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. *Science* **302**, 1972-1975.
7. Montes de Oca Luna, R., Wagner, D. S. and Lozano, G. (1995) Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. *Nature* **378**, 203-206.
8. Claret, F. X., Hibi, M., Dhut, S., Toda, T. and Karin, M. (1996) A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. *Nature* **383**, 453-457.
9. Tomoda, K., Kubota, Y. and Kato, J. (1999) Degradation of the cyclin-dependent-kinase inhibitor p27Kip1 is instigated by Jab1. *Nature* **398**, 160-165.
10. Doronkin, S., Djagaeva, I. and Beckendorf, S. K. (2003) The COP9 signalosome promotes degradation of Cyclin E during early Drosophila oogenesis. *Dev. Cell* **4**, 699-710.
11. Kim, B. C., Lee, H. J., Park, S. H., Lee, S. R., Karpova, T. S., McNally, J. G., Felici, A., Lee, D. K. and Kim, S. J. (2004) Jab1/CSN5, a component of the COP9 signalosome, regulates transforming growth factor beta signaling by binding to Smad7 and promoting its degradation. *Mol. Cell Biol.* **24**, 2251-2262.
12. Wan, M., Cao, X., Wu, Y., Bai, S., Wu, L., Shi, X., Wang, N. and Cao, X. (2002) Jab1 antagonizes TGF-beta signaling by inducing Smad4 degradation. *EMBO Rep.* **3**, 171-176.
13. Callige, M., Kieffer, I. and Richard-Foy, H. (2005) CSN5/Jab1 is involved in ligand-dependent degradation of estrogen receptor {alpha} by the proteasome. *Mol. Cell Biol.* **25**, 4349-4358.
14. Bech-Otschir, D., Kraft, R., Huang, X., Henklein, P., Kapelari, B., Pollmann, C. and Dubiel, W. (2001) COP9 signalosome-specific phosphorylation targets p53 to degradation by the ubiquitin system. *Embo J.* **20**, 1630-1639.
15. Oh, W., Lee, E. W., Sung, Y. H., Yang, M. R., Ghim, J., Lee, H. W. and Song, J. (2006) Jab1 induces the cytoplasmic localization and degradation of p53 in coordination with Hdm2. *J. Biol. Chem.* **281**, 17457-17465.
16. Lee, E. W., Oh, W. and Song, J. (2006) Jab1 as a mediator of nuclear export and cytoplasmic degradation of p53. *Mol. Cells* **22**, 133-140.
17. Tomoda, K., Yoneda-Kato, N., Fukumoto, A., Yamanaka, S. and Kato, J. Y. (2004) Multiple functions of Jab1 are required for early embryonic development and growth potential in mice. *J. Biol. Chem.* **279**, 43013-43018.
18. Fukumoto, A., Ikeda, N., Sho, M., Tomoda, K., Kanehiro, H., Hisanaga, M., Tsurui, Y., Tsutsumi, M., Kato, J. Y. and Nakajima, Y. (2004) Prognostic significance of localized p27Kip1 and potential role of Jab1/CSN5 in pancreatic cancer. *Oncol. Rep.* **11**, 277-284.
19. Goto, A., Niki, T., Moriyama, S., Funata, N., Moriyama, H., Nishimura, Y., Tsuchida, R., Kato, J. Y. and Fukayama, M. (2004) Immunohistochemical study of Skp2 and Jab1,

- two key molecules in the degradation of P27, in lung adenocarcinoma. *Pathol. Int.* **54**, 675-681.
20. Ito, Y., Yoshida, H., Matsuzuka, F., Matsuura, N., Nakamura, Y., Nakamine, H., Kakudo, K., Kuma, K. and Miyauchi, A. (2003) Jun activation domain-binding protein 1 expression in malignant lymphoma of the thyroid: its linkage to degree of malignancy and p27 expression. *Anticancer Res.* **23**, 4121-4125.
  21. Korbonits, M., Chahal, H. S., Kaltsas, G., Jordan, S., Urmanova, Y., Khalimova, Z., Harris, P. E., Farrell, W. E., Claret, F. X. and Grossman, A. B. (2002) Expression of phosphorylated p27 (Kip1) protein and Jun activation domain-binding protein 1 in human pituitary tumors. *J. Clin. Endocrinol. Metab.* **87**, 2635-2643.
  22. Kouvaraki, M. A., Rassidakis, G. Z., Tian, L., Kumar, R., Kittas, C. and Claret, F. X. (2003) Jun activation domain-binding protein 1 expression in breast cancer inversely correlates with the cell cycle inhibitor p27 (Kip1). *Cancer Res.* **63**, 2977-2981.
  23. Shintani, S., Li, C., Mihara, M., Hino, S., Nakashiro, K. and Hamakawa, H. (2003) Skp2 and Jab1 expression are associated with inverse expression of p27 (KIP1) and poor prognosis in oral squamous cell carcinomas. *Oncology* **65**, 355-362.
  24. Sui, L., Dong, Y., Ohno, M., Watanabe, Y., Sugimoto, K., Tai, Y. and Tokuda, M. (2001) Jab1 expression is associated with inverse expression of p27 (kip1) and poor prognosis in epithelial ovarian tumors. *Clin. Cancer Res.* **7**, 4130-4135.
  25. Shen, L., Tsuchida, R., Miyauchi, J., Saeki, M., Honna, T., Tsunematsu, Y., Kato, J. and Mizutani, S. (2000) Differentiation-associated expression and intracellular localization of cyclin-dependent kinase inhibitor p27KIP1 and c-Jun co-activator JAB1 in neuroblastoma. *Int. J. Oncol.* **17**, 749-754.