Adenovirus-mediated expression of p33^{ING1b} induces apoptosis and inhibits proliferation in gastric adenocarcinoma cells in vitro

Yifei Lv · Bibek Kumar Purbey · Yanhua Huang ·
Shuang Li · Gurung Radha · Zhiming Hao

Received: 12 July 2011 / Accepted: 26 November 2011 / Published online: 12 January 2012
© The International Gastric Cancer Association and The Japanese Gastric Cancer Association 2011

Abstract

Background Inhibitor of growth 1b (ING1b) is considered to be a class II tumor suppressor gene. Although reduced expression of p33^{ING1b} has been reported in many human malignancies, including gastric cancers, the effect of p33^{ING1b} on gastric cancer cells has yet to be investigated.

Methods Expression of p33^{ING1b} in gastric adenocarcinoma tissues and their adjacent non-malignant gastric mucosa, as well as in gastric adenocarcinoma cell lines and normal gastric epithelial cells, was detected by using Western blotting. Recombinant adenoviruses were prepared to mediate the ectopic expression of p33^{ING1b} (Ad-ING1b) and green fluorescent protein (GFP)(Ad-GFP) in the gastric adenocarcinoma cell lines, SGC-7901, MKN28, and MKN45 and the normal gastric epithelial cell line GES-1. Alterations in the proliferation and apoptosis of the cells after adenoviral infection were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and flow cytometry, respectively, and cell cycle distribution was analyzed in a fluorescence-activated cell sorter.

Results Western blotting confirmed the reduced expression of p33^{ING1b} in gastric adenocarcinoma tissues and gastric adenocarcinoma cell lines. The ectopic expression of p33^{ING1b} mediated by Ad-ING1b resulted in decreased growth, increased apoptosis, and cell cycle arrest at the G1

phase in both benign and malignant gastric epithelial cells regardless of their p53 status. Addition of a p53 inhibitor, pifithrin- α , did not abolish the pro-apoptotic and cell cycle-arresting effects of p33^{ING1b} in p53 wild-type cells.

Conclusions Down-regulation of p33^{ING1b} might play an important role in the development of gastric adenocarcinoma. Targeted local expression of p33^{ING1b} may offer a promising alternative therapeutic measure for gastric cancer.

Keywords Gastric adenocarcinoma · p33^{ING1b} · Proliferation · Cell cycle · Apoptosis

Introduction

Over the past few decades, substantial progress has been made in defining potential molecular pathways in carcinogenesis and identifying many new potential therapeutic targets. Molecular mechanisms underlying carcinogenesis involve abnormalities of oncogenes, tumor suppressor genes (TSGs), cell adhesion molecules, cell cycle regulators, DNA repair, genetic instability, and growth factors and cytokines. Inhibition of growth 1 (ING1), a newly identified TSG, has been proposed to take part in the concert of carcinogenesis [1].

The ING1 gene is localized to chromosome 13q34 and encodes at least 4 protein isoforms (p47^{ING1a}, p33^{ING1b}, p24^{ING1c}, and p27^{ING1d}) as a result of the usage of different promoters, exons, and alternative splicing [2]. All of these isoforms share an identical C-terminus containing a plant homeodomain (PHD) zinc finger motif that binds histone H3 in a methylation-sensitive manner to take part in chromatin remodeling, and a nuclear localization signal (NLS) which drives them to enter and reside in the nucleus

Y. Lv and B. K. Purbey contributed equally to this work.

Y. Lv · B. K. Purbey · Y. Huang · S. Li · G. Radha ·
Z. Hao (✉)

Department of Gastroenterology, 1st Affiliated Hospital, School
of Medicine, Xi'an Jiaotong University, 710061 Xi'an, China
e-mail: haozhm@yahoo.com.cn

[1, 2]. Among these variants, p33^{ING1b} is the most intensively studied and the most extensively expressed isoform in human tissues. Studies have revealed that p33^{ING1b} is expressed in almost all normal tissues and cells, suggesting a fundamental and pivotal function(s) of p33^{ING1b} [3]. Apart from the above-mentioned domains, p33^{ING1b} also harbors three other domains: a proliferating cell nuclear antigen (PCNA) interacting protein motif (PIP) domain which binds PCNA following UV irradiation and is involved in apoptosis and cell cycle arrest, a lamin interaction domain (LID) which binds lamin A/HDAC complex to maintain its levels and biological function in the nucleus, and a partial bromodomain (PBD) which is commonly found in chromatin-associated protein [4]. Recently, it was found that serine 199 of p33^{ING1b} could be phosphorylated and by this site p33^{ING1b} could bind to 14-3-3 family proteins, resulting in the translocation of p33^{ING1b} from the nucleus to the cytoplasm [5]. Studies have demonstrated that p33^{ING1b} participates in growth regulation, senescence, DNA repair, apoptosis, and angiogenesis [6]. Based on reports of malignancies in the stomach [7], esophagus [8], brain [9], breast [10], blood [11, 12], lung [13], liver [14], head and neck [15], lymphoid system [16], thyroid [17], and ovary [18], it can be deduced that the expression of p33^{ING1b} is often reduced in human malignancies, while mutations in ING1b occur infrequently [19]. Blocking ING1 expression promotes cell proliferation in vitro and tumor formation in vivo [20]. Restoration of p33^{ING1b} function has been shown to stabilize the malignant phenotype of neoplastic cells. For example, adenovirus-mediated ING1b gene transfer significantly suppressed the growth and promoted the apoptosis of glioma cells in vitro [21].

Although reduced expression of ING1b mRNA has been reported in gastric adenocarcinoma [7], the role of p33^{ING1b} in the development and biological behaviors of gastric adenocarcinoma is far from elucidated. In the present study, we firstly confirmed the reduced expression of p33^{ING1b} in gastric adenocarcinoma tissues and gastric adenocarcinoma cell lines, and then, by using recombinant adenovirus-mediated ectopic expression, we observed the effects of p33^{ING1b} on proliferation, cell cycle distribution, and apoptosis in gastric adenocarcinoma cells.

Methods

Patients and tissue specimen preparation

Gastric adenocarcinoma samples and their adjacent non-malignant tissues (at least 2 cm away from the tumor) were collected endoscopically from eight patients at the 1st Affiliated Hospital, School of Medicine, Xi'an Jiaotong

University, after obtaining approval from the Institutional Review Boards of Xi'an Jiaotong University, Xi'an. The diagnosis of gastric adenocarcinoma was confirmed histologically. The samples were immediately snap-frozen in liquid nitrogen and stored at -80°C .

Cell culture

SGC-7901, MKN28, and MKN45 (gastric adenocarcinoma cell lines), GES-1 (a normal gastric epithelial cell line), and Ad-293 (adenovirus packaging cells) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL), penicillin (100 U/ml), and streptomycin (100 $\mu\text{g}/\text{ml}$) at 37°C in a humidified CO_2 (5%) atmosphere. Cells were passaged at 1:3 every 3 days.

Western blotting

Gastric tissues and cultured cells were homogenized in RIPA lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% sodium dodecylsulfate [SDS], 0.02% sodium azide, 1% nonidet P-40, and 0.5% sodium deoxycholate) containing protease inhibitors (100 $\mu\text{g}/\text{ml}$ phenylmethanesulfonyl fluoride [PMSF], 1 $\mu\text{g}/\text{ml}$ aprotinin), on an ice-bath, followed by centrifugation at 12,000 rpm for 20 min at 4°C . Protein concentration was determined by Bradford assay. Each protein sample (100 μg) was separated on a 10% SDS-polyacrylamide gel (PAGE) and transferred onto a nitrocellulose membrane (Millipore, Bedford, MA, USA). The membrane was blocked with blocking buffer (5% nonfat dry milk and 0.2% Tween-20 in phosphate-buffered saline [PBS]) and probed with a primary antibody against p33^{ING1b} (polyclonal antibody prepared in our laboratory by immunizing Balb/c mice with 6 $\times$ His-tagged N-terminal fragment of human p33^{ING1b} expressed in *Escherichia coli*, 1:400 dilution) or β -actin (1:1000 dilution; Sigma, St Louis, MO, USA), followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG as secondary antibody (1:2,000 dilution; Sigma). The blots were detected using an enhanced chemiluminescence solution and exposing the membranes to Kodak X-ray film (Kodak, Rochester, NY, USA).

Preparation of recombinant adenoviruses

Recombinant adenovirus (type 5, $\Delta\text{E1}/\text{E3}$) carrying ING1b was prepared by using the AdeasyTM Adenovirus Vector System (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. Briefly, cDNA of p33^{ING1b} was isolated from pCI-ING1b (kindly provided by Dr. K.T. Riabowol, University of Calgary, Canada) by *Xho*I and *Sma*I digestion and subcloned into pShuttle-CMV. The

recombinant shuttle plasmid pShuttle-CMV-ING1b was linearized by digestion with the restriction endonuclease PmeI (New England BioLabs, Ipswich, MA, USA) and then transformed into *E. coli* BJ5183 containing pAdEasy-1, yielding the recombinant adenoviral plasmid pAd-ING1b. After digestion by *PacI* (New England Biolabs), the ~23-kb fragment of pAd-ING1b was recovered and transfected into Ad-293 cells to produce recombinant adenovirus Ad-ING1b. Aliquots of viral stocks were stored at -80°C until use. Adenovirus expressing green fluorescent protein (Ad-GFP) was also prepared as a control. The viral stocks were titrated on HEK293 cells by a standard protocol just as the manufacturer instructed.

For determination of the appropriate multiplicity of infection (MOI), SGC-7901, MKN28, MKN45, and GES-1 cells were inoculated in 6-well plates and infected with Ad-GFP at MOI of 20, 40, 60, 80, and 100. Seventy-two hours later, the cells were observed under an inverted fluorescence microscope to evaluate the infection efficiency and possible toxic effects.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cells were inoculated into 96-well micro-culture plates at 1×10^3 /well, grown overnight, and infected with Ad-GFP or Ad-ING1b at an MOI of 60. The MTT assay was carried out to assess the number of viable cells before and post-infection, at 24-h intervals. Upon determination, 20 μl MTT was added to each well, followed by incubation at 37°C for 4 h. Then the MTT-containing media was removed and 150 μl dimethylsulfoxide (DMSO) was added to each well. Absorbance at 490 nm was measured using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). This experiment was performed in triplicate wells and three independent experiments were carried out.

Detection of apoptosis

Apoptosis was determined by flow cytometry (FCM) using an ApoScreen Annexin V-APC kit (Southernbiotech, Birmingham, AL, USA). Cells were seeded into six-well tissue culture plates at a density of 1×10^5 cells/well and infected with recombinant adenoviruses at an MOI of 60. To verify whether the effect of p33^{ING1b} on apoptosis was p53-dependent, a p53 inhibitor, pifithrin- α (PFT- α , Sigma-Aldrich, St. Louis, MO, USA) was added to MKN-45 and GES-1 cells to reach a final concentration of 20 μmol at the time of infection. Seventy-two hours later, cells were harvested by trypsinization, washed with cooled PBS at 4°C , and centrifuged at 1,500 rpm for 5 min. The pellet was then resuspended in 100 μl binding buffer (10 mM

hydroxyethylpiperazine ethanesulfonic acid [HEPES], pH 7.4, 140 mM NaCl, 2.5 mM CaCl_2 , 0.5% bovine serum albumin [BSA]) and mixed gently with 10 μl of APC-labeled Annexin V and 10 μl of propidium iodide (PI). After incubation in the dark at 4°C for 10 min, the sample was mixed with 380 μl $1\times$ binding buffer and vortexed gently. Apoptosis was analyzed in a flow cytometer.

Cell cycle analysis

Twelve hours after the inoculation into 6-well plates, the cells were cultured in DMEM supplemented with 0.2% bovine serum (serum starvation) for 24 h to achieve cell cycle synchronization. Then the cells were infected with adenoviruses and harvested just as in the case of detection of apoptosis. The harvested cells were washed once with ice-cold PBS and fixed with 70% ethanol at 4°C for 12 h. After two washes with ice-cold PBS, the cells were treated with RNase for 30 min and stained with 50 mg/ml of PI. Cell cycle distribution was analyzed in a fluorescence-activated cell sorter (Becton–Dickinson, Mountain View, CA, USA).

Statistical analysis

Results are presented as means $\pm$ standard deviation ($M \pm SD$). Comparisons among means for more than two groups were performed using one-way analysis of variance (ANOVA). Unpaired *t*-test was used for comparison between two groups. Results were considered statistically significant at $P < 0.05$. All analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Expression of p33^{ING1b} was down-regulated in gastric adenocarcinoma tissues and gastric adenocarcinoma cell lines

Western blotting showed that all the paratumor gastric mucosa specimens exhibited p33^{ING1b} expression. However, gastric adenocarcinoma tissues showed an overall (7 out of 8 specimens) decreased expression level of p33^{ING1b} compared with paratumor tissues (Fig. 1a). Just as in the case of the gastric tissue specimens, the expression of p33^{ING1b} in the three gastric adenocarcinoma cell lines (SGC-7901, MKN28, and MKN45) was lower than that in the normal gastric epithelial cell line, GES-1 (Fig. 1b). All these results clearly indicate that the expression of p33^{ING1b} is down-regulated in gastric adenocarcinoma.

Fig. 1 Reduced expression of p33^{ING1b} in gastric adenocarcinoma tissues and cell lines assessed by Western blotting. **a** Expression of p33^{ING1b} in gastric adenocarcinoma and their adjacent paratumor tissues. **b** Expression of p33^{ING1b} in gastric adenocarcinoma cell lines and the normal gastric epithelial cell line, GES-1

Adenovirus could efficiently infect malignant and benign gastric epithelial cells and mediate ectopic p33^{ING1b} expression

At an MOI of 60, more than 60% of the cells expressed GFP and at that time no obvious adenoviral toxicity was observed (Fig. 2a). Thus subsequently in this study, cells were infected by recombinant adenoviruses at an MOI of 60. Next, Western blotting verified the ectopic expression of p33^{ING1b} mediated by Ad-ING1b in gastric cells (Fig. 2b). The above results indicate that adenovirus could efficiently mediate gene transfer and ectopic expression in all the cells used in this study.

Ectopic expression of p33^{ING1b} inhibited in vitro growth of gastric adenocarcinoma cells and normal gastric epithelial cells

The MTT assay showed that all the four lines of cells infected with Ad-ING1b displayed obviously lower growth rates than their corresponding controls, including the Ad-GFP and PBS groups (Fig. 3). From day 4 onwards, the numbers of viable cells in the Ad-ING1b groups were significantly lower than those in both the Ad-GFP and PBS

Fig. 2 Infection efficiency of recombinant adenovirus (Ad) to gastric adenocarcinoma cells and normal gastric epithelial cells. **a** Fluorescence of the cells 72 h after Ad-green fluorescent protein (GFP) infection ($\times 100$). **b** Western blot detection of ectopic expression of p33^{ING1b} in the cells 72 h after Ad-ING1b infection

groups, while no significant difference in growth rate was observed between the latter two groups. These results indicate that the growth inhibition of Ad-ING1b could be attributed to the ectopic expression of p33^{ING1b}, and the toxic effect of adenovirus could be excluded.

Ectopic expression of p33^{ING1b} induced apoptosis of gastric adenocarcinoma and normal gastric epithelial cells

To define the effect of Ad-ING1b on apoptosis in gastric adenocarcinoma cells and normal gastric epithelia, the cells were analyzed by FCM 72 h after infection. All the cells exhibited a low spontaneous apoptosis rate when treated with PBS. After Ad-GFP infection, the apoptosis rates increased slightly. Infection with Ad-ING1b resulted in a

Fig. 3 Inhibitory effect of ectopic p33^{ING1b} on the proliferation of gastric adenocarcinoma cells and normal gastric epithelial cells. The results are presented as means $\pm$ SD from three independent experiments. * $P < 0.05$ versus Ad-GFP and phosphate-buffered saline (PBS; control) groups at the same time point

significant increase in apoptosis rates in all the four cell lines. In the Ad-ING1b group, apoptosis rates more than eightfold those in the Ad-GFP and PBS groups were observed. For MKN45 and GES-1 cells, which harbor wild-type p53, no significant difference in apoptosis rates was observed between the Ad-ING1b group and the Ad-ING1b plus PFT- α group (Fig. 4).

p33^{ING1b} arrested cell cycle progression at G1 phase in gastric adenocarcinoma and normal gastric epithelial cells

To explore the possible mechanism underlying the growth-inhibitory effect of p33^{ING1b}, the cell cycle distribution in SGC-7901 and GES-1 cells 72 h post-infection was analyzed. Both SGC-7901 and GES-1 cells infected with Ad-ING1b demonstrated a significant increase in cell numbers at the G1 stage compared with those infected with Ad-GFP or uninfected (PBS group) cells ($P < 0.01$), while no significant difference was observed in cell cycle distribution between cells infected with Ad-GFP and uninfected cells. In addition, no significant difference in cell cycle distribution was discerned between the Ad-ING1b group and the Ad-ING1b plus PFT- α group in GES-1 cells (Fig. 5).

Discussion

p33^{ING1b} has been shown to play an important role in multiple cellular processes such as DNA repair, proliferation, and apoptosis regulation via modulating the transcription of downstream biological molecules [20]. The normal expression level and correct subcellular localization of p33^{ING1b} is essential for a body or cell to maintain homeostasis. Down-regulated expression, mutation, or mislocalization of p33^{ING1b} leads to the uncontrolled growth of cells and is involved in carcinogenesis [20]. Reduced p33^{ING1b} expression, regardless of whether it occurs via mutation or mislocalization, is present in many cancers. A previous study showed reduced expression of ING1b mRNA in gastric adenocarcinoma, suggesting that down-regulation of ING1b might participate in gastric carcinogenesis [7]. However, the expression of p^{33ING1b} at the protein level has not been reported. In the present study, we detected the expression of p33^{ING1b} in gastric adenocarcinoma specimens and gastric cancer cell lines by using Western blotting. The results showed that the expression of p33^{ING1b} in gastric carcinoma tissues was obviously reduced compared with that in their paired paratumor gastric mucosa. Also, p33^{ING1b} expression was down-regulated in the gastric adenocarcinoma cell lines SGC-7901, MKN28, and MKN45 compared with the normal

Fig. 4 p33^{ING1b} induced apoptosis in SGC-7901, MKN28, MKN45, and GES-1 cells. **a** Representative dot plot profiles of flow cytometry (FCM) analysis. **b** Results of FCM analysis for the detection of

apoptosis. Cells were treated and analyzed as indicated in “Methods”. * $P < 0.05$ versus Ad-GFP and PBS groups. § $P > 0.05$ versus Ad-ING1b group. PFT- α Pifithrin- α

gastric epithelial cell line GES-1. Although the cellular localization of p33^{ING1b} in gastric adenocarcinoma tissues was not investigated in our study, studies on malignancies of other origins with immunohistochemistry have demonstrated reduced overall expression of p33^{ING1b} accompanied

by nucleus-to-cytoplasm translocation in cancer cells [11, 22]. Based on these results, it can be concluded that decreased expression of p33^{ING1b} is a common phenomenon in gastric adenocarcinoma, and this alteration might contribute to the development of gastric adenocarcinoma.

Fig. 5 p33^{ING1b} induced cell cycle arrest at G1 phase. **a** Representative figures of FCM cytometric detection of cell cycle distribution. **b** The results of cell cycle distribution analysis. MKN45 and GES-1 cells were treated and cell cycle distribution was analyzed by FCM as described in “Methods”. * $P < 0.05$ versus Ad-GFP and PBS groups.

§ $P > 0.05$ versus Ad-ING1b group. p33^{ING1b} is down-regulated in gastric carcinoma tissues and gastric adenocarcinoma cell lines. Adenovirus-mediated ectopic expression of p33^{ING1b} suppresses proliferation and induces apoptosis in gastric adenocarcinoma cells

Next, we observed the influence of the ectopic expression of p33^{ING1b} on the proliferation, apoptosis, and cell cycle distribution of gastric adenocarcinoma and normal gastric epithelial cells. To guarantee the efficiency of gene transfer, we prepared recombinant adenovirus to mediate the expression of ING1b. The results showed that the ectopic expression of p33^{ING1b} induced growth retardation, cell cycle arrest at the G1 phase, and, especially, apoptosis, in both the malignant and normal gastric epithelial cells in vitro. Our present results are supported by the previous studies in which p33^{ING1b} exhibited proliferation-inhibitory, apoptosis-promoting, and cell cycle-arrest effects in both benign and malignant cells [20–29]. These data, along with the results of reduced expression of p33^{ING1b} in gastric adenocarcinoma tissues, provide substantial evidence that p33^{ING1b} acts as a negative regulator in the development of gastric adenocarcinoma.

p33^{ING1b} was formerly considered to exert its biological functions through physical and functional interaction with p53 [21, 28, 29]. Recently, some studies demonstrated that

p33^{ING1b} could function in a p53-independent manner [25, 30, 31]. Our present results revealed that p33^{ING1b} could induce growth retardation, apoptosis, and cell cycle arrest in both p53 wild-type (MKN45, GES-1) and p53 mutant (MKN28, SGC-7901) cells. Furthermore, for p53 wild-type cells, the addition of the p53 inhibitor pifithrin- α did not abolish the pro-apoptosis and cell cycle-arrest effects of p33^{ING1b}. These results suggest that the pro-apoptosis and anti-proliferation effects of p33^{ING1b} are, at least partly, independent of p53.

To our knowledge, this is the first study to have explored the possible role and mechanisms of p33^{ING1b} in the biological behaviors of gastric carcinoma. Our results confirmed the down-regulated expression of p33^{ING1b} in human gastric adenocarcinoma and demonstrated the anti-proliferation and pro-apoptosis effects of Ad-ING1b in human gastric adenocarcinoma cells regardless of their p53 status. These results suggest that targeted local administration of Ad-ING1b might be considered as a promising therapeutic measure for gastric adenocarcinoma.

Acknowledgments We thank Dr. K. T. Riabowol, University of Calgary, Canada, for providing the pCI-ING1b plasmid.

References

- Ythier D, Larrieu D, Brambilla C, Brambilla E, Pedoux R. The new tumor suppressor genes ING: genomic structure and status in cancer. *Int J Cancer*. 2008;123:1483–90.
- Garkavtsev I, Demetrick D, Riabowol K. Cellular localization and chromosome mapping of a novel candidate tumor suppressor gene (ING1). *Cytogenet Cell Genet*. 1997;76:176–8.
- Jäger D, Stockert E, Scanlan MJ, Güre AO, Jäger E, Knuth A, et al. Cancer-testis antigens and ING1 tumor suppressor gene product are breast cancer antigens: characterization of tissue-specific ING1 transcripts and a homologue gene. *Cancer Res*. 1999;59:6197–204.
- Han X, Feng X, Rattner JB, Smith H, Bose P, Suzuki K, et al. Tethering by lamin A stabilizes and targets the ING1 tumour suppressor. *Nat Cell Biol*. 2008;10:1333–40.
- Gong W, Russell M, Suzuki K, Riabowol K. Subcellular targeting of p33ING1b by phosphorylation-dependent 14-3-3 binding regulates p21WAF1 expression. *Mol Cell Biol*. 2006;26:2947–54.
- Gong W, Suzuki K, Russell M, Riabowol K. Function of the ING family of PHD proteins in cancer. *Int J Biochem Cell Biol*. 2005;37:1054–65.
- Oki E, Maehara Y, Tokunaga E, Kakeji Y, Sugimachi K. Reduced expression of p33(ING1) and the relationship with p53 expression in human gastric cancer. *Cancer Lett*. 1999;147:157–62.
- Chen L, Matsubara N, Yoshino T, Nagasaka T, Hoshizima N, Shirakawa Y, et al. Genetic alterations of candidate tumor suppressor ING1 in human esophageal squamous cell cancer. *Cancer Res*. 2001;61:4345–9.
- Tallen G, Riabowol K, Wolff JE. Expression of p33^{ING1} mRNA and chemosensitivity in brain tumour cells. *Anticancer Res*. 2003;23:1631–5.
- Toyama T, Iwase H, Watson P, Muzik H, Saettler E, Magliocco A, et al. Suppression of ING1 expression in sporadic breast cancer. *Oncogene*. 1999;18:5187–93.
- Nouman GS, Anderson JJ, Crosier S, Shrimankar J, Lunec J, Angus B. Downregulation of nuclear expression of the p33^{ING1b} inhibitor of growth protein in invasive carcinoma of the breast. *J Clin Pathol*. 2003;56:507–11.
- Ito K, Kinjo K, Nakazato T, Ikeda Y, Kizaki M. Expression and sequence analyses of p33 (ING1) gene in myeloid leukemia. *Am J Hematol*. 2002;69:141–3.
- Kameyama K, Huang CL, Liu D, Masuya D, Nakashima T, Sumitomo S, et al. Reduced ING1b gene expression plays an important role in carcinogenesis of non-small cell lung cancer patients. *Clin Cancer Res*. 2003;9:4926–34.
- Ohgi T, Masaki T, Nakai S, Morishita A, Yukimasa S, Nagai M, et al. Expression of p33 (ING1) in hepatocellular carcinoma: relationships to tumour differentiation and cyclin E kinase activity. *Scand J Gastroenterol*. 2002;37:1440–8.
- Gunduz M, Ouchida M, Fukushima K, Hanafusa H, Etani T, Nishioka S, et al. Genomic structure of the human ING1 gene and tumor-specific mutations detected in head and neck squamous cell carcinomas. *Cancer Res*. 2000;60:3143–6.
- Ohmori M, Nagai M, Tasaka T, Koeffler HP, Toyama T, Riabowol K, et al. Decreased expression of p33^{ING1b} mRNA in lymphoid malignancies. *Am J Hematol*. 1999;62:118–9.
- Nouman GS, Angus B, Lunec J, Crosier S, Lodge A, Anderson JJ. Comparative assessment expression of the inhibitor of growth 1 gene (ING1) in normal and neoplastic tissues. *Hybrid Hybridomics*. 2002;21:1–10.
- Shen DH, Chan KYK, Khoo US, Ngan HYS, Xue WC, Chiu PM, et al. Epigenetic and genetic alterations of p33^{ING1b} in ovarian cancer. *Carcinogenesis*. 2005;26:855–63.
- Tallen G, Kaiser I, Krabbe S, Lass U, Hartmann C, Henze G. No ING1 mutations in human brain tumours but reduced expression in high malignancy grades of astrocytoma. *Int J Cancer*. 2004;109:476–9.
- Garkavtsev I, Kazarov A, Gudkov A, Riabowol K. Suppression of the novel growth inhibitor p33^{ING1} promotes neoplastic transformation. *Nat Genet*. 1996;14:415–20.
- Shinoura N, Muramatsu Y, Nishimura M, Yoshida Y, Saito A, Yokoyama T, et al. Adenovirus-mediated transfer of p33^{ING1} with p53 drastically augments apoptosis in gliomas. *Cancer Res*. 1999;59:5521–8.
- Nouman GS, Anderson JJ, Mathers ME, Leonard N, Crosier S, Lunec J, Angus B. Nuclear to cytoplasmic compartment shift of the p33^{ING1b} tumour suppressor protein is associated with malignancy in melanocytic lesions. *Histopathology*. 2002;40:360–6.
- Goeman F, Thormeyer D, Abad M, Serrano M, Schmidt O, Palmero I, et al. Growth inhibition by the tumor suppressor p33^{ING1} in immortalized and primary cells: involvement of two silencing domains and effect of Ras. *Mol Cell Biol*. 2005;25:422–31.
- Garkavtsev I, Riabowol K. Extension of the replicative life span of human diploid fibroblasts by inhibition of the p33(ING1) candidate tumor suppressor. *Mol Cell Biol*. 1997;17:2014–9.
- Tsang FC, Po LS, Leung KM, Lau A, Siu WY, Poon RY. ING1b decreases cell proliferation through p53-dependent and -independent mechanisms. *FEBS Lett*. 2003;553:277–85.
- Zhu Z, Luo Z, Li Y, Ni C, Li H, Zhu M. Human inhibitor of growth 1 inhibits hepatoma cell growth and influences p53 stability in a variant-dependent manner. *Hepatology*. 2009;49:504–12.
- Garate M, Campos EI, Bush JA, Xiao H, Li G. Phosphorylation of the tumor suppressor p33(ING1b) at Ser-126 influences its protein stability and proliferation of melanoma cells. *FASEB J*. 2007;21:3705–16.
- Zhu Z, Lin J, Qu JH, Feitelson MA, Ni CR, Li FM, et al. Inhibitory effect of tumor suppressor p33^{ING1b} and its synergy with p53 gene in hepatocellular carcinoma. *World J Gastroenterol*. 2005;11:1903–9.
- Zhu JJ, Li FB, Zhu XF, Liao WM. The p33^{ING1b} tumor suppressor cooperates with p53 to induce apoptosis in response to etoposide in human osteosarcoma cells. *Life Sci*. 2006;78:1469–77.
- Cheung KJ Jr, Bush JA, Jia W, Li G. Expression of the novel tumour suppressor p33(ING1) is independent of p53. *Br J Cancer*. 2000;83:1468–72.
- Coles AH, Liang H, Zhu Z, Marfella CGA, Kang J, Imbalzano AN, et al. Deletion of p37Ing1 in mice reveals a p53-independent role for Ing1 in the suppression of cell proliferation, apoptosis, and tumorigenesis. *Cancer Res*. 2007;67:2054–61.