

# Enhanced anti-melanoma efficacy of interferon $\alpha$ -2b via overexpression of ING4 by enhanced Fas/FasL-mediated apoptosis

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**Abstract.** Melanoma, is a highly aggressive and the most lethal form of skin cancer, and is known to be resistant to current therapeutic modalities. Interferon (IFN)- $\alpha$ 2b is an immunostimulatory cytokine and is used to treat melanoma by inhibiting proliferation and promoting apoptosis of cells. However, there is a need to improve the efficacy of IFN- $\alpha$ 2b. Inhibitor of growth family member 4 (ING4) has been reported to function as a tumor suppressor and is involved in regulating cell cycle progression, apoptosis, cell migration and invasion. Previously studies have also reported that caspase-3, caspase-8, poly (ADP-ribose) polymerase (PARP) and Fas/Fas ligand (FasL) pathways are involved in the process of apoptosis. In the present study, it was investigated whether overexpression of ING4 is able to enhance IFN- $\alpha$ 2b response in human melanoma cells. It was determined that the overexpression of ING4 was able to increase the effects of IFN- $\alpha$ 2b, and induce cell death and apoptosis in melanoma cells. Furthermore, the overexpression of ING4 resulted in decreased expression of PARP, caspase-3 and -8. The expression of cleaved PARP, cleaved caspase-3, cleaved caspase-8, Fas and FasL was increased in the A375 melanoma cell line. These results demonstrate that the overexpression of ING4 is able to enhance the anti-melanoma activity of IFN- $\alpha$ 2b. These findings provide a potential therapeutic strategy where a combination of ING4 overexpression and IFN- $\alpha$ 2b treatment may lead to higher levels of apoptosis in melanoma cells.

## Introduction

Malignant melanoma is the severe and life threatening cancer of melanocytes, which has increased by ~3% steadily throughout the last decade (1). Malignant melanoma is considered curable when detected at an early stage. However, once malignant melanoma has entered the advanced stages of disease, it disseminates widely and becomes an incurable malignancy with a very poor prognosis (2). Due to special characteristics, including a large intercellular distance, poorly defined cell borders and architectural disarray (3), and usual resistance to standard chemotherapy, there is no systemic and effective therapy at present that has a clear effect on the overall survival of patients with malignant melanoma (4). Although the understanding of the molecular biology of malignant melanoma has increased in recent years, the molecular mechanism of melanomagenesis is not completely understood and requires further elucidation.

Although cytotoxic chemotherapy is widely used in patients with metastatic melanoma, it now has a limited role with little to no response in patients (5). Historically, patients with metastatic melanoma that were treated with conventional chemotherapies have a poor prognosis, with median survival duration of merely 6-9 months (6,7). Recombinant interferon (IFN)- $\alpha$  has been utilized in the treatment of patients with malignant melanoma following surgery (8). Clinically relevant benefits from IFN- $\alpha$  therapy include an increase in the duration of disease-free survival (DFS) by 9 months and also an increase in the rate of 5-year progression-free survival by 9% (9,10).

It has been indicated that the efficacy of IFN- $\alpha$  therapy requires improvement (11). IFN- $\alpha$  regulates the anti-proliferative effects on melanoma cells and modulates the immune response (12). Melanoma cells are less responsive to IFN- $\alpha$  compared with immune cells (13). Specifically, IFN- $\alpha$  has been shown to sensitize malignant cells to increase the expression of death receptors and by upregulating the expression of cell cycle regulatory proteins, including p21, which is a cyclin-dependent kinase inhibitor (14,15). These data suggested that IFN- $\alpha$  therapy potentially serves a role in increasing the pro-apoptotic effects in melanoma.

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The Fas receptors and Fas ligands (Fas/FasL) system is a key regulator of apoptosis in T-cells (16). In early- or intermediate-phase melanomas, the Fas apoptotic pathway is active. However, the Fas apoptotic pathway is altered in highly metastatic melanomas (17). Furthermore, the lack of Fas expression in malignant melanomas is associated with a poor prognosis (18). During the progression of melanoma, the expression of Fas is decreased and the expression of FasL is increased (19).

Inhibitor of growth family member 4 (ING4), which is a member of the conserved ING family, has been identified as a novel tumor suppressor (20). ING4, which is located on chromosome 12p13, contains two nuclear localization signals and a highly conserved plant homeodomain finger motif at the C-terminal end (21). The expression of ING4 is significantly decreased in primary and metastatic melanomas compared with dysplastic nevi (22). Furthermore, the overexpression of ING4 resulted in a diminished colony-forming efficiency, a decreased S phase cell population, and apoptosis was induced in a p53-dependent manner (21,22).

In the present study, it was hypothesized that ING4 is able to enhance the anti-tumor effects of IFN- $\alpha$ 2b by activating the common pro-apoptotic pathways. In the present study, it was demonstrated that the combination of overexpression of ING4 and IFN- $\alpha$ 2b treatment in malignant melanoma cells was able to induce apoptosis. Apoptosis induced by this combination treatment was associated with a decreased expression of caspase-3, and -8 as well as poly (ADP-ribose) polymerase (PARP). Additionally, the combination treatment also resulted in an increased expression of cleaved caspase-3, cleaved caspase-8 and cleaved PARP. Notably, the combination of ING4 overexpression and IFN- $\alpha$ 2b treatment was able to effectively induce apoptosis, which resulted in increased Fas/FasL death signaling. Increased Fas/FasL death signaling was associated with cellular survival and resistance to apoptosis in melanoma cells.

The results of the present study indicate that the effect of overexpression of ING4 was increased in the presence of IFN- $\alpha$ 2b, and this may be a potential treatment strategy for melanoma.

## Materials and methods

**Cell culture.** The human melanoma cell lines A375 and HT-144 were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), containing 10% (v/v) fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at 37°C in a humidified incubator with 5% CO<sub>2</sub>.

**Construction of ING4 overexpression or knockdown vectors in human melanoma cells.** The pcDNA3.1-ING4 was constructed as previously described (23). The lentiviral vector (pLKO.1) constructs containing ING4 cDNA or 100  $\mu$ M short hairpin RNA sequences (LV-ING4-RNAi: 5'-GCTTGCCATGCAGACCTATGA-3') for ING4 and empty vector were purchased from Open Biosystems (Thermo Fisher Scientific, Inc.). Lentiviral constructs were used to transiently transfect HEK293 packaging cells along with VSV-G pseudoviral

particles. Virus-containing medium was collected from HEK293 cells and filtered to remove non-adherent cells 2 and 3 days post-transfection. Subconfluent A375 or HT-144 cells were incubated with virus-containing medium and 8  $\mu$ g/ml polybrene. Infected A375 or HT-144 cells were selected starting at 24 h after initial infection using 2  $\mu$ g/ml puromycin. The titer of LV-ING4-RNAi or LV-ING4 were  $\sim 5 \times 10^7$  IU/ml, and the multiplicity of infection (MOI) was 5, where >80% of cells were identified to be green fluorescent protein (GFP)-positive. Infected A375 or HT-144 cells were passaged at least 3 times every 3-5 days prior to further experiments. The protein levels in cells were analyzed by western blot analysis as described below.

**Cell viability assay.** The human melanoma cell lines A375 and HT-144 were trypsinized and dispensed in 96-well plates at a density of  $1 \times 10^5$  cells/well. After incubation at various concentrations of IFN $\alpha$ 2b (0,  $5 \times 10^3$ ,  $5 \times 10^4$ ,  $5 \times 10^5$  and  $5 \times 10^6$  IU/l) for 24 or 48 h at 37°C, 50  $\mu$ l MTT (1 mg/ml; Merck KGaA) was added to cell media. Following incubation for 4 h at 37°C, MTT was discarded, and 150  $\mu$ l DMSO was loaded in each well. The spectrophotometric absorbance of the samples was measured using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 570 nm with a reference wavelength of 655 nm. The experiments were performed at least three times, and three wells were measured at each concentration.

**Analysis of apoptosis with Annexin V/7-aminoactinomycin D (7-AAD) staining.** Apoptosis was detected using Annexin V-phycoerythrin/7-AAD staining (Apoptosis Detection kit; cat. no. KGA1017; Kaiji Inc, Nanjing, China; <http://www.keygentec.com.cn/prd-search-KGA1017.html>). Human melanoma cell line A375 was suspended and transferred to a sterile culture tube, 5  $\mu$ l Annexin V-phycoerythrin and 5  $\mu$ l 7-AAD were added to each tube. The tubes were gently vortexed and incubated for 15 min at room temperature in the dark. Fluorescence-activated cell sorting (FACS) analysis was performed using the BD FACSort flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Cell populations were classified into four groups: Viable (Annexin V<sup>-</sup>, 7-AAD<sup>-</sup>), early apoptotic (Annexin V<sup>+</sup>, 7-AAD<sup>-</sup>), late apoptotic (Annexin V<sup>+</sup>, 7-AAD<sup>+</sup>) or necrotic (Annexin V<sup>-</sup>, 7-AAD<sup>+</sup>). Data were analyzed using CellQuest software (BD Biosciences, version 5.1).

**Western blot analysis.** Human melanoma cell line A375 was rinsed in PBS, then the cell pellets were re-suspended in lysis buffer [137 mM NaCl, 1% NP-40, 50 mmol/l Tris (pH 8.0), and 20% glycerol] and normalized to total protein concentration using Bio-Rad protein assay. Whole cell proteins (30  $\mu$ g) were separated on 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes using the BioRad electrotransfer system (Bio-Rad Laboratories, Inc.). The membranes were incubated with antibodies against ING4 (cat. no. sc-135742), PARP (cat. no. sc-136208), caspase 8 (cat. no. sc-6136), caspase 3 (cat. no. sc-271759), Fas (cat. no. sc-4856), FasL (cat. no. sc-71096) (dilution, 1:1,000; Santa Cruz Biotechnology, Inc.) and cleaved PARP (cat. no. 5625), anti-cleaved caspase-8 (cat. no. 8529) and cleaved caspase-3 (cat. no. 9661) (dilution,

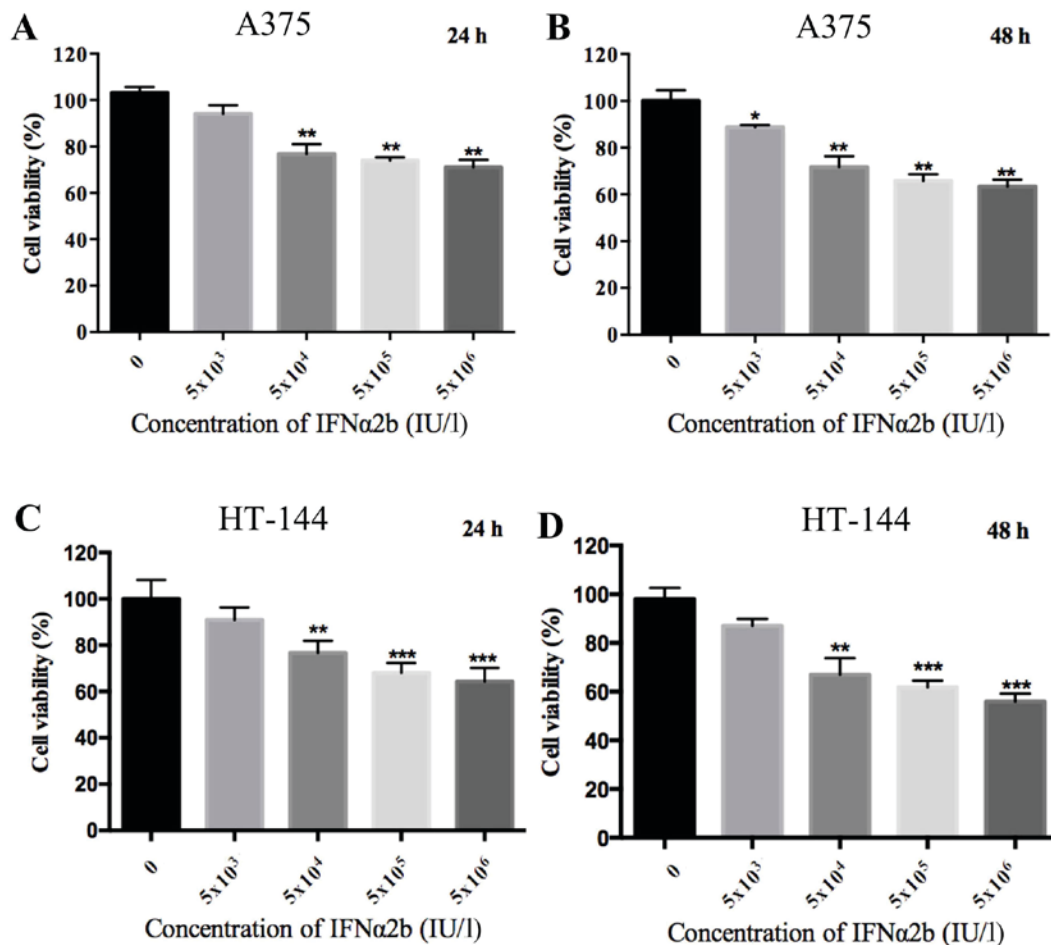


Figure 1. IFN- $\alpha$ 2b inhibits the survival and proliferation of melanoma cells. A375 cells were treated with (0, 5x10<sup>3</sup>, 5x10<sup>4</sup>, 5x10<sup>5</sup> and 5x10<sup>6</sup> IU/l) IFN- $\alpha$ 2b for (A) 24 h or (B) 48 h. HT-144 cells were treated with (0, 5x10<sup>3</sup>, 5x10<sup>4</sup>, 5x10<sup>5</sup> and 5x10<sup>6</sup> IU/l) IFN- $\alpha$ 2b for (C) 24 or (D) 48 h. The treated cells were incubated with MTT, and the number of viable cells was analyzed spectrophotometrically at 570 nm. The bars represent the mean  $\pm$  standard deviation of triplicate values for three independent experiments. \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001. IFN, interferon.

1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA) for 1 h at room temperature. GAPDH (cat. no. sc-47724, dilution, 1:1,000; Santa Cruz Biotechnology, Inc.) was used as a loading control. The membrane was blocked using 5% fat-free milk (Sigma-Aldrich; Merck KGaA) for 1 h at room temperature and then incubated with the appropriate primary antibody diluted in 3% BSA solution (Sigma-Aldrich; Merck KGaA) at 4°C overnight. Following incubation with goat anti-mouse secondary antibody (cat. no., 35518; 1:10,000 dilution; Thermo Fisher Scientific, Inc.) and goat anti-rabbit secondary antibody (cat. no., A32731; 1:10,000 dilution; Thermo Fisher Scientific, Inc.) for 1 h at room temperature. The blots were incubated by the Odyssey Western Blotting kit (LI-COR Biosciences, Lincoln, NE, USA) and scanned using the Odyssey Western Detection system (LI-COR Biosciences, Lincoln, NE, USA), followed by quantification with ImageStudio software (LI-COR Biosciences; version 5.2.5) as previously described (24).

**Statistical analysis.** Data are presented as the mean  $\pm$  standard deviation. Prism (version 6.0; GraphPad Software, Inc., La Jolla, CA, USA) was used to perform statistical analysis. Statistical significance between two groups was determined using an unpaired Student's t-test. Comparisons among multiple groups were determined by one-way or two-way

analysis of variance followed by Dunnett's or Bonferroni's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

## Results

**Overexpression of ING4 increases the anti-cancer effect of IFN $\alpha$ 2b in human melanoma cells.** To assess whether the expression of ING4 enhances the anti-cancer effect of IFN $\alpha$ 2b, the effects of various concentrations of IFN $\alpha$ 2b (0, 5x10<sup>3</sup>, 5x10<sup>4</sup>, 5x10<sup>5</sup> and 5x10<sup>6</sup> IU/l) on the survival of melanoma cells were examined by MTT assay. As shown in Fig. 1, a dose-dependent decrease in the viability of melanoma cells was observed with increasing concentrations of IFN $\alpha$ 2b (P<0.01). However, the melanoma cell viability in the high concentrations of IFN $\alpha$ 2b (5x10<sup>5</sup> and 5x10<sup>6</sup> IU/l) was similar to the 5x10<sup>4</sup> IU/l IFN $\alpha$ 2b group.

To elucidate the function of ING4 in melanoma cells, ING4 was either knocked down or overexpressed in A375 or HT-144 cells using LV-ING4-RNAi or LV-ING4, and the expression of ING4 was examined by western blotting. The results indicated that downregulation of ING4 was achieved with LV-ING4-RNAi, and LV-ING4 markedly increased the expression of ING4 (Fig. 2A). The ability of ING4 to act

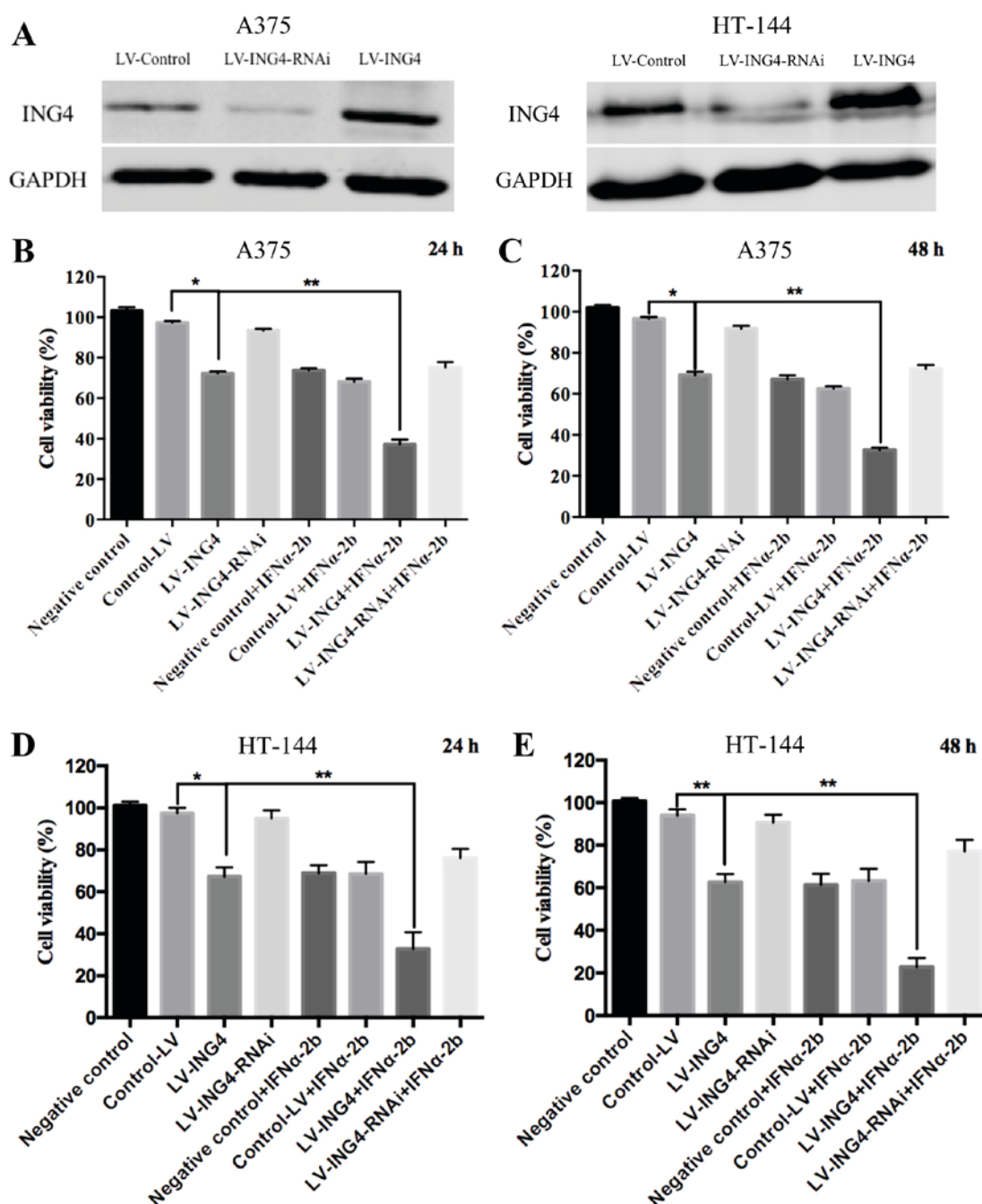


Figure 2. Synergistic effects of ING4 and IFN- $\alpha$ 2b expression on A375 cells. (A) The expression of ING4 at 48 h following transfection with LV-control, LV-ING4-RNAi or LV-ING4 in A375 cells (left panel) or HT-144 cells (right panel). A375 cells were treated with LV-control, LV-ING4-RNAi or LV-ING4 for 48 h. The cells were subsequently treated with  $5 \times 10^4$  IU/l IFN- $\alpha$ 2b for (B) 24 or (C) 48 h. HT-144 Cells were treated with LV-control, LV-ING4-RNAi or LV-ING4 for 48 h, then treated with  $5 \times 10^4$  IU/l IFN- $\alpha$ 2b for (D) 24 or (E) 48 h. Cell viability was examined by MTT assay. \* $P < 0.05$ , \*\* $P < 0.01$ .

synergistically with IFN $\alpha$ 2b to induce cell death of A375 or HT-144 was analyzed by MTT assay. As shown in Fig. 2B-E, the overexpression of ING4 alone decreased the cell viability to ~70% when compared with control-LV (~97%) for 24 or 48 h. However, IFN $\alpha$ 2b treatment increased the cytotoxic effect. The overexpression of ING4 reduced the viability of IFN $\alpha$ 2b-treated A375 cells (concentration of IFN $\alpha$ 2b  $5 \times 10^4$  IU/l) from  $70 \pm 1.75$  to  $32 \pm 1.05\%$ . These results showed that the overexpression of ING4 in melanoma cells led to significantly increased inhibition on cell growth compared with IFN $\alpha$ 2b treatment alone. However, the downregulation of ING4 alone had no effect on the viability of melanoma

cells (Fig. 2). The results illustrated that the overexpression of ING4 is able to increase the anti-cancer effects of IFN $\alpha$ 2b in different types of melanoma cells (Figs. 1 and 2).

*Combination treatment of A375 cell lines with overexpression of ING4 and IFN- $\alpha$ 2b leads to increased apoptosis of melanoma cells.* A combination of ING4 overexpression and IFN- $\alpha$ 2b treatment was indicated to induce apoptosis in A375 cells compared with single treatments (Fig. 3). Consequently, it was hypothesized that the pro-apoptotic effects of a combination of ING4 overexpression and IFN- $\alpha$ 2b treatment would be greater compared with single treatments in melanoma cell lines.



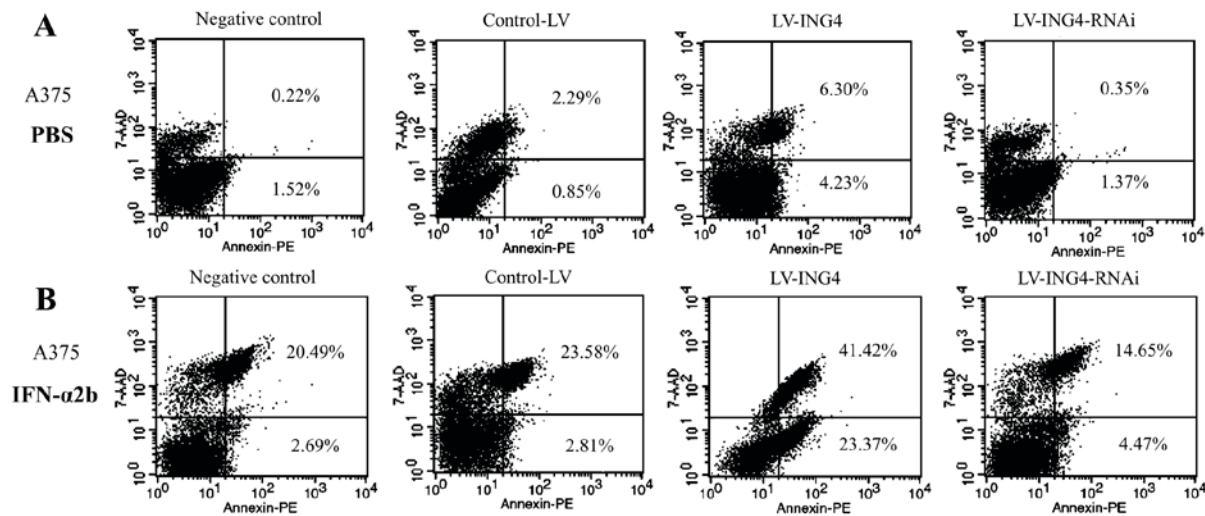


Figure 3. Combination of ING4 and IFN- $\alpha$ 2b overexpression increases the apoptosis of A375 melanoma cell lines more markedly compared with overexpression of either gene alone. Fluorescence-activated cell sorting analysis of A375 cells at 48 h following transfection with LV-control, LV-ING4-RNAi or LV-ING4. A375 cells were stained with Annexin V-fluorescein isothiocyanate and 7-AAD following transfection with LV-control, LV-ING4-RNAi or LV-ING4. The cells were either treated (A) without or (B) with  $5 \times 10^4$  IU/l IFN- $\alpha$ 2b. The percentages represent the proportion of cells that are Annexin V-positive/7-AAD-negative (early apoptotic) and Annexin V-positive/7-AAD-positive cells (apoptotic). IFN, interferon; ING4, inhibitor of growth family member 4; PBS, phosphate buffered saline; PE, phycoerythrin; 7-AAD, 7-aminoactinomycin D.

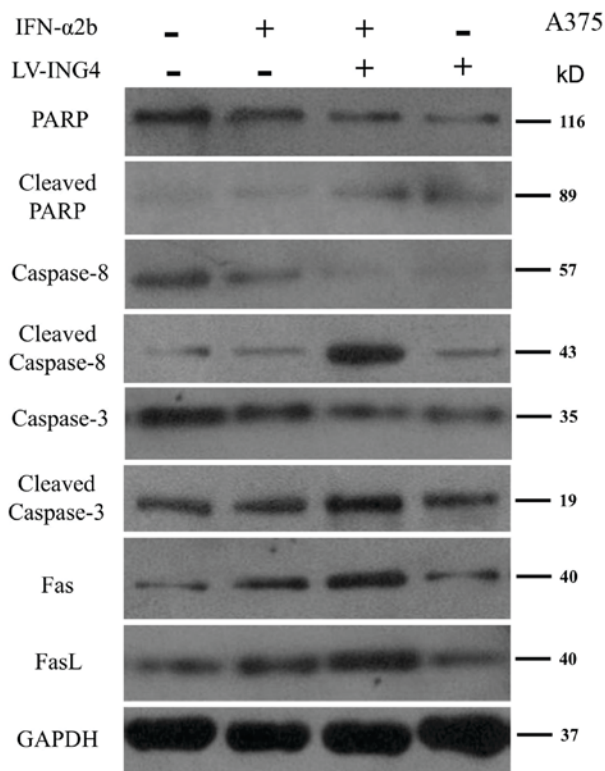


Figure 4. Western blot analysis of PARP, cleaved PARP, caspase 8, cleaved caspase 8, caspase 3, cleaved caspase 3, Fas and FasL in A375 cells that were treated with PBS, IFN- $\alpha$ 2b, a combination of IFN- $\alpha$ 2b and LV-ING4 or LV-ING4 alone. ING4, inhibitor of growth family member 4; PARP, poly(ADP-ribose) polymerase; FasL, Fas ligand; IFN, interferon.

The transfection of LV-ING4 or LV-ING4-RNAi was able to induce apoptosis in A375 cells, as assessed by FACS analysis. The percentage of early apoptotic cells (Annexin V<sup>+</sup>/7-AAD<sup>-</sup>) was 1.52% for control, 0.85% for LV-control, 4.23% for LV-ING4 and 1.37% for the LV-ING4-RNAi group. Furthermore, the

percentage of late apoptotic cells (Annexin V<sup>+</sup>/7-AAD<sup>+</sup>) was 0.22% for control, 2.29% for LV-control, 6.30% for LV-ING4 and 0.35% for LV-ING4-RNAi (Fig. 3A).

In order to determine whether ING4 is able to synergize with IFN $\alpha$ 2b to induce the apoptosis of A375 cells, the cells were treated with LV-ING4 or LV-ING4-RNAi and IFN- $\alpha$ 2b. As indicated in Fig. 3B, the combination of ING4 overexpression and IFN- $\alpha$ 2b treatment induced a greater level of apoptosis in the A375 cells compared with single treatments (Fig. 3B). The percentage of early apoptotic cells of the IFN- $\alpha$ 2b-treated A375 cells that were transfected with LV-ING4 was 23.37%, and the percentage of late apoptotic cells was 41.42%. By contrast, the percentage of early apoptotic cells in the LV-control group that was treated with IFN- $\alpha$ 2b was 2.81%, and the percentage of late apoptotic cells was 23.58%. Therefore, there is a predominantly apoptotic effect in the combined ING4 overexpression and IFN- $\alpha$ 2b group in A375 cells, compared with the LV-control combined with IFN- $\alpha$ 2b group.

*Combination of ING4 overexpression and treatment of IFN- $\alpha$ 2b induces the effector caspases and Fas/FasL.* The mechanisms underlying the effect of IFN- $\alpha$ 2b treatment and ING4 overexpression on the apoptosis of A375 cells was investigated. The expression of a number of proteins that are involved in cell apoptosis were detected, including PARP, cleaved PARP, caspase-3, cleaved caspase-3, caspase-8, cleaved caspase-8, Fas and FasL. As shown in Fig. 4, the expression of PARP, caspase-3 and caspase-8 were markedly decreased in A375 cells that were treated with a combination of IFN- $\alpha$ 2b and LV-ING4 compared with the cells that were treated with IFN- $\alpha$ 2b alone. Furthermore, the expression of cleaved PARP, cleaved caspase-3 and cleaved caspase-8 were increased in A375 cells that were treated with a combination of IFN- $\alpha$ 2b and LV-ING4 compared with the cells that were treated with IFN- $\alpha$ 2b alone (Fig. 4).

## Discussion

The majority of patients with melanoma have a poor response to IFN- $\alpha$ , which limits the clinical benefits of IFN- $\alpha$ 2b as a therapy (25). In the present study, the overexpression of ING4 was indicated to enhance the anti-tumor activity of IFN- $\alpha$ 2b in A375 and HT-144 melanoma cells and induce cell apoptosis, which was associated with PARP, caspase-8 and caspase-3. In addition, the treatment of melanoma cells with a combination of ING4 overexpression and IFN- $\alpha$ 2b was indicated to result in an increased level of Fas/FasL protein (Fig. 4). Therefore, ING4 may be a critical positive regulator of the anti-tumor effect of IFN- $\alpha$ 2b in melanoma, and combination treatment with ING4 overexpression and IFN- $\alpha$ 2b may serve a role in the treatment of melanoma.

ING4 is a strong candidate for a tumor suppressor gene within the ING family due to its important role in a variety of cellular processes, including oncogenesis, angiogenesis, gene transcription, cell cycle and apoptosis (20,21,26). As a nuclear factor, ING4 is widely expressed in normal human tissues, but its expression is significantly decreased in various types of cancer, including breast tumors, gliomas, and squamous cell carcinomas (20,26). Furthermore, a previous study identified that the expression of ING4 is significantly reduced in human malignant melanomas (22), suggesting that ING4 serves an important role in melanoma tumorigenesis. Additionally, the growth of tumor cells was significantly inhibited following the transfection of exogenous ING4 by interfering with cell cycle progression in the human lung adenocarcinoma cell line A549.

Apoptosis or programmed cell death consists of the ordered disassembly of the cell from within as opposed to necrosis or accidental cell death. Apoptosis may be induced through the extrinsic pathway, which involves the activation of cell surface death receptors, or the intrinsic pathway, where alterations in the integrity of the mitochondrial membrane induce the release of cytochrome *c* (27). These pathways converge at the level of the effector caspases (caspase-3, -6, -7 and -8) (28). Once activated, these effector caspases cleave cytoskeletal and nuclear proteins, such as PARP, thereby initiating cellular disassembly (28). The results of the present study demonstrated that the expression of PARP, caspase-8 and caspase-3 is decreased in response to the treatment of melanoma cells with a combination of ING4 and IFN- $\alpha$ 2b, supporting the hypothesis that cell death occurs in a manner consistent with apoptosis.

The cell death pathway mediated via Fas/FasL interaction represents typical apoptotic signaling in various cell types (29). In many cell types and tissues, including HepG2, U87MG and HeLa cells, Fas and its receptor, FasL, and caspase-8 are components of an important cellular pathway that regulates the induction of apoptosis (30). In the present study, the data indicated that treatment with a combination of IFN- $\alpha$ 2b and LV-ING4 markedly activated Fas and FasL proteins (Fig. 4). Future studies can examine the activity of the cleaved PARP, cleaved caspase-8 and cleaved caspase-3, which recapitulates key features of apoptosis.

In addition to its immune-enhancing effects, IFN- $\alpha$  is also known to act on tumor cells to decrease cellular proliferation and promote apoptosis (31). These processes are mediated in part via the transcription and translation

of IFN- $\alpha$  stimulated genes (32). In the present study, the pre-transfection of melanoma cells with ING4 and treatment with IFN- $\alpha$ 2b was demonstrated to increase the expression of Fas/FasL. This data suggests that the overexpression of ING4 may enhance the sensitivity of melanoma cells to the direct effects of IFN- $\alpha$ . IFN- $\alpha$  is one of the few treatment options that are available to those afflicted with malignant melanoma, and these suggest that there may be methods to enhance the activity of IFN- $\alpha$ .

IFN- $\alpha$ 2b has various effects on cancer cells. Notably, the combination of IFN- $\alpha$ 2b treatment and ING4 overexpression may be particularly useful as it was demonstrated in the present study that IFN- $\alpha$ 2b is able to induce the upregulation of Fas/FasL. Additionally, the combination of LV-ING4 and IFN- $\alpha$ 2b treatment was able to significantly increase the apoptotic effect in the examined melanoma cell lines compared with single treatments (Fig. 2), suggesting that the overexpression of ING4 may be effective as part of a combination therapy regimen for advanced melanomas. It will be interesting to test the effects of ING4 overexpression and IFN- $\alpha$ 2b treatment in primary cancer cells obtained from patients with metastatic melanoma.

In summary, the present study suggests that a combination of ING4 overexpression and IFN- $\alpha$ 2b treatment may be a novel treatment strategy for inducing direct, apoptotic effects on human melanoma cells.

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## Availability of data and materials

Not applicable.

## Authors' contributions

LMC, JL, YCW and YW conceived and designed the experiments. LMC, JL, HXC and YM performed the experiments and analyzed data. LMC, JL, YW, HXC, YM, YW and YCW contributed reagents, materials, and/or analysis tools. YCW, LMC and JL wrote the main manuscript text. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that there are no conflicts of interest.

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