# Oridonin increases anticancer effects of lentinan in HepG2 human hepatoblastoma cells

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**Abstract.** The aim of the present study was to investigate whether oridonin is able to increase the effects of lentinan (LNT) in HepG2 human hepatoblastoma cells by MTT, flow cytometry, reverse transcription-quantitative polymerase chain reaction and western blot analysis. The *in vitro* results demonstrated that 20 μg/ml of oridonin was a nontoxic concentration for L02 normal liver cells and HepG2 liver cancer cells. Furthermore, treatment with 0-200 μg/ml LNT was only able to decrease the viability of HepG2 liver cancer cells. The growth inhibitory rate of the LNT-L (100 μg/ml) treatment group was 20.7% and the rate of the LNT-H (200 μg/ml) treatment group was 54.8%. Notably, the growth inhibitory rate of the oridonin + LNT-H group was 84.3%. The highest percentage of apoptotic cells was observed in the oridonin + LNT-H group (20 μg/ml oridonin and 200 μg/ml LNT). The percentage of apoptotic cells in the oridonin + LNT-H group was significantly different from the percentage of apoptotic cells in the LNT-H (26.1%) and the LNT-L (16.8%) groups. Treatment with LNT produced an increase in caspase-3, caspase-9, Bcl-2-like protein 4, p53, p21, nuclear factor κB inhibitor-α mRNA and protein expression and a decrease in B-cell lymphoma 2 and nuclear factor-κB expression in HepG2 cells compared with untreated control cells. Treatment with a combination of oridonin and LNT-H induced a further increase in expression with the biggest differences in expression observed between the oridonin + LNT-H group and control. It was observed that treatment with oridonin was able to increase the anticancer effects of LNT in HepG2 cells. Therefore, oridonin may be used to sensitize cells to LNT.

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## Introduction

Lentinan (LNT) is a type of medicinal polysaccharide isolated from shiitake with various activities including, immune regulation, anti-tumor, anti-virus and anti-infection effects. LNT has high efficacy and limited side effects (1). LNT has good curative effects on gastric, colon, breast and lung cancer, which is able to prolong the survival time of patients with tumor (2). LNT is often used as an immune enhancer in clinical application, which can enhance curative effects or reduce side effects in combination with other drugs (2). Murata *et al* (3) reported that a combination of cisplatin and lentinan significantly increased the anti-cancer activity in the treatment of colon cancer. Drandarska *et al* (4) demonstrated that treatment with LNT is able to increase the activation of Bacillus Calmette-Guérin (BCG)-induced pulmonary macrophages in guinea pigs and reduce systemic adverse reactions of BCG vaccine.

Oridonin is an ent-kaurene diterpenoid compound mainly isolated from *R. rubescen* (5). Previous studies have reported that oridonin is able to promote tumor cell apoptosis (3,6). Apoptotic rate has been hypothesized to have an effect on the sensitivity of tumors to radiation (7). A previous study also indicated that the sensitization effect of oridonin may enhance the efficacy of radiotherapy in liver cancer cell lines (8).

An ideal cancer therapeutic would be a drug that can induce differentiation and apoptosis of tumor cells. Previous clinical studies have focused on Chinese medicine preparations based on anti-proliferative effects, while in recent years the focus has shifted to the development of preparations which can induce differentiation and apoptosis of cancer cells (9,10). Drugs that induce apoptosis can selectively target cancer cells and therefore normal cells are unaffected by treatment (9).

A number of active chemical substances in natural plants have strong apoptotic-inducing effects on cancer cells (8-10), and studies have demonstrated that lentinan and oridonin are active substances with cancer cell apoptosis-inducing effects (3,8).

Substances that can induce apoptosis in cancer cells, which are extracted from plants have a low level of toxicity and can be safely used. These substances are also able to relieve pain in the process of treatment. However, the efficacy of numerous cancer inhibitor components in plants is much lower compared with synthetic drugs (11). Therefore, a combination of different

natural substances can increase the inhibitory and therapeutic effects (11).

Finding a reasonable combination is an important aspect of the research in the anti-cancer activities of natural products. By studying how oridonin is able to increase the anticancer effect of lentinan *in vitro*, the present study aimed to verify the effects of a novel combination of anti-cancer substances. By using MTT assay, flow cytometry, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blotting, the present study investigated the effect of oridonin treatment on growth inhibition hepatoblastoma cells *in vitro*. The study also investigated the effect of lentinan treatment on the apoptosis of hepatoblastoma cells, in order to accumulate further data that may enable future animal experiments and even clinical application.

**Materials and methods**

*Cell lines and treatments*. Human normal liver L02 cell lines and human hepatoblastoma HepG2 cells were purchased from the Conservation Genetics CAS Kunming Cell Bank (Kunming, China). Oridonin and lentinan were purchased from Shanghai Shamrock Imp and Exp Trading Co., Ltd. (Shanghai, China). Lentinan-Low (LNL-L, 100 $\mu$g/ml) and Lentinan-High (LNL-H, 200 $\mu$g/ml) treatment groups were generated for the two cell lines.

*Cell culture*. The normal human liver L02 cell lines (control group) and human hepatoblastoma HepG2 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and cultured in an incubator under humidified atmosphere of 5% $CO_2$ at 37°C. The medium was changed 2-3 times a week and sub-cultured for 6-7 days. Subsequently, the cells were seeded in a 96-well culture plate at a density of $1x10^4$/ml with 180 $\mu$l per well, and cultured for 24 h under humidified atmosphere of 5% $CO_2$ at 37°C.

*MTT assay*. The L02 (control group) and HepG2 cells were incubated with Oridonin (20 $\mu$g/ml) and Lentinan (100 $\mu$g/ml) for 24 h at room temperature, respectively. After 24 h, 20 $\mu$l MTT solution (5 mg/ml; Ameresco Inc., Framingham, MA, USA) was added to each well and incubated at 37°C for 4 h, following which the culture medium was replaced with 150 $\mu$l dimethyl sulfoxide (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The absorbance was measured at 540 nm using a microplate spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following formula was used to calculate the inhibitory rate: Percentage cell viability=[(Absorbance of untreated cells-absorbance of treated cells)/absorbance of untreated cells] x100 (12).

*Flow cytometry analysis of cell cycle distribution and apoptosis*. HepG2 cells were seeded at a density of $50x10^4$ cells/60-mm dish and incubated overnight at 37°C. Oridonin was added to a final concentration of 40 $\mu$M and cells were incubated for 24 h at 37°C. The cells were treated as follows: i) 20 $\mu$g/ml oridonin + 200 $\mu$g/ml lentinan; ii) PBS (negative control); iii) 100 $\mu$g/ml lentinan; and iv) 200 $\mu$g/ml lentinan. Detached and adherent

cells were collected and centrifuged at 1,500 x g for 5 min at 4°C. Pellets were rinsed with ice-cold PBS and fixed with 70% ethanol at 4°C overnight. The density of HepG2 cells was adjusted to $5x10^5$ cells/ml, and the cells were washed with PBS three times. Cells were subsequently stained with staining buffer (PBS containing 20 $\mu$g/ml of propidium iodide, 100 $\mu$g/ml RNase A and 0.1% Triton X-100; BD Biosciences, Franklin Lakes, NJ, USA) for 15 min at 4°C in the dark. The cells were subsequently labeled with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (Annexin V-FITC apoptosis detection kit; BD Biosciences; cat. no. 556547). Samples were analyzed using a flow cytometer (BD Biosciences) and Cell Quest acquisition software (version 2.9; BD Biosciences) (13).

*Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay*. Cells from different treatment groups (20 $\mu$g/ml oridonin + 200 $\mu$g/ml lentinan; PBS as negative control; 100 $\mu$g/ml lentinan; and 200 $\mu$g/ml lentinan) were collected, and total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using a cDNA reverse transcription kit (cat. no., 1708840; Bio-Rad Laboratories, Inc.) according to manufacturer's protocol. The resultant DNA (10 $\mu$l) was subjected to a 25 $\mu$l PCR conducted in an iCycler thermal cycler (Bio-Rad Laboratories, Inc.) using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc.). The thermocycling conditions that were used were as follows: Initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 20 sec, 58°C for 20 sec and 72°C for 20 sec, with a final extension of 72°C for 5 min. β-actin was used as the internal reference gene. The relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (14) and gene expression was normalized to β-actin. The primers used in the present study were as follows: B-cell lymphoma (Bcl)-2 forward, 5'-CAAAGGTGGATCAGATTCAAG-3'; Bcl-2 reverse, 5'-GGTGAGCATTATCACCCAGAA-3'; Bcl-2-associated protein X (Bax) forward, 5'-TGGCAGCAGTGACAGCAG CG-3'; Bax reverse, 5'-TACGGAGGTGGAGTGGGTGT-3'; caspase-3 forward, 5'-AAAGTTTTCAATGACCAAGC-3'; caspase-3 reverse, 5'-TCTGACGAATCTCCTCCAC-3'; caspase-9 forward, 5'-AGTCTATTTTATTATGGGCTCG-3'; caspase-9 reverse, 5'-TGGATGTTTATGTCACCTTTTC-3'; p21 forward, 5'-ATGGAGAACACTGAAAACTC-3'; p21 reverse, 5'-TGTGAGCATGGAAACAATAC-3'; p53 forward, 5'-ACTCCCATTCTTCCACCTTTG-3'; p53 reverse, 5'-CCC TGTTGCTGTAGCCATATT-3'; nuclear factor κB (NF-κB) forward, 5'-GCTATTCAGGCTGTGCTGTC-3'; NF-κB reverse, 5'-GGTAGTCGGTGAGATCTCGG-3'; nuclear factor κB inhibitor α (IκB-α) forward, 5'-CCAACTATT GCTTCAGCTCCA-3' IκB-α reverse, 5'-GTGTCCAGGCTC CAAATGT-3'; β-actin forward, 5'-AGCCTTCTCCATGGT CGTGA-3'; and β-actin reverse, 5'-CGGAGTCAACGGATT TGGTC-3'. Primers were synthesized by Invitrogen; Thermo Fisher Scientific, Inc.

*Western blot analysis*. HepG2 cells treated with oridonin and/or lentinan as aforementioned were homogenized and lysed with radioimmunoprecipitation assay lysis buffer (Invitrogen; Thermo Fisher Scientific, Inc.; 100 mM NaCl,
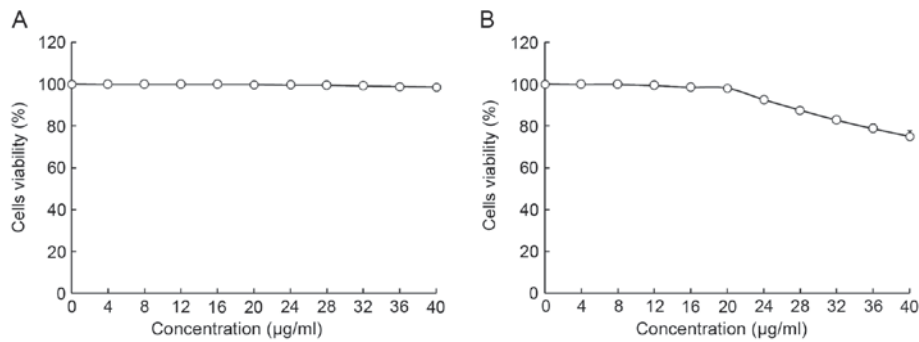
Figure 1. Effect of oridonin treatment on the viability of human normal liver cells (A) L02 and (B) HepG2 human hepatoblastoma cells as determined by MTT assay.
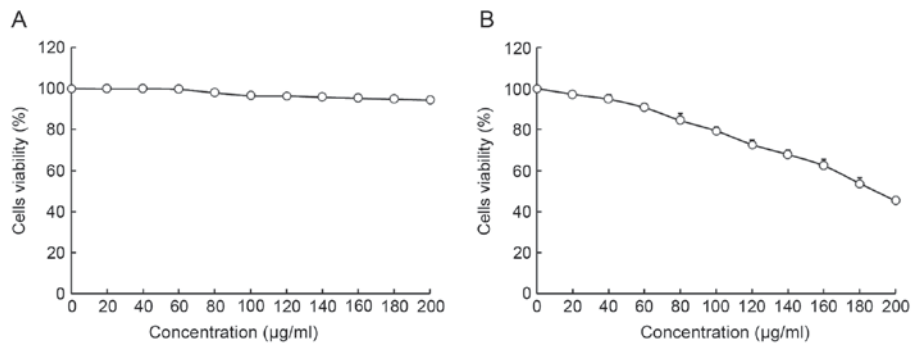


Figure 2. Effect of lentinan treatment on the viability of human normal liver cells (A) L02 and (B) HepG2 human hepatoblastoma cells as determined by MTT assay.

50 mM Tris-HCl pH 7.5, 1% Triton X-100, 1 mM EDTA, 10 mM β-glycerophosphate, 2 mM sodium vanadate and protease inhibitor). Lysates were sonicated for 5 sec on ice and centrifuged at 6,000 x g for 5 min at 4°C. Supernatants were collected and the protein concentration was detected using a Bio-Rad protein assay kit (cat. no. 500-0002; Bio-Rad Laboratories, Inc.). A total of 20 µg protein/well was loaded to SDS-PAGE (10% gel; GE Healthcare Life Sciences, Shanghai, China) and transferred onto polyvinylidene difluoride membranes, which were activated by methanol. The membrane was blocked using 10% skimmed milk at room temperature for 1 h. Subsequently, the membranes were incubated with the primary antibodies against caspase-3 (1:1,000; cat. no. ab13847), caspase-9 (1:1,000; cat. no. ab18571), Bcl-2 (1:1,000; cat. no. ab194583), Bax (1:1,000; cat. no. ab32503), p38 (1:1,000; cat. no. ab31828), p53 (1:1,000; cat. no. ab1431), NF-κB (1:1,000; cat. no. ab32360), IκB-αα (1:1,000; cat. no. ab7217) and β-actin (1:5,000; cat. no. ab8226), all purchased from Abcam (Cambridge, UK), overnight at 4°C. The following day, the membranes were washed with TBST for 10 min, prior to incubation with the horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:1,000; cat. no. ab6728; Abcam) for 1 h at room temperature and washed three times with TBST (10 min per wash). The membranes were visualized using ECL chemiluminescence agent, and β-actin was used as a control for normalization. Immunoreactivity was determined using enhanced chemiluminescent reagent (Thermo Fisher Scientific, Inc.) using an ImageQuant Las4000 digital imager (Thermo Fisher Scientific, Inc.).

*Statistical analysis.* Data are presented as the mean ± standard deviation. Statistical evaluation was performed using Student's t-test or one-way analysis of variance followed by Student-Newman-Keuls test using SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Effects of oridonin and lentinan treatment on L02 and HepG2 cells.* The viability of HepG2 and L02 cells following treatment with oridonin or LNT was determined using MTT assay (Figs. 1 and 2). Treatment with 0-20 µl/ml oridonin did not result in a decrease in viability in L02 or HepG2 cells (Fig. 1A and B). It was observed that LNT treatment was able to decrease the viability of HepG2 cells at an increased concentration. Treatment with 0-200 µl/ml LNT was able to decrease the viability of HepG2 cancer cells. However, treatment with the same concentration of LNT did not result in a decrease in viability in L02 cells.

Based on these results, 20 µl/ml oridonin was selected for subsequent experiments to investigate whether oridonin treatment is able to increase the anti-cancer effect of LNT. Concentrations of 100 and 200 µl/ml were selected to verify the anticancer effects of LNT (Fig. 2).

The growth inhibition values of HepG2 human hepatoblastoma cells by oridonin and lentinan are presented in Table I. The highest $OD_{540}$ value was observed in the HepG2 cancer cells; oridonin + LNT-H (20 µg/ml oridonin + 200 µg/ml LNT), LNT-H (200 µg/ml LNT) and LNT-L (100 µg/ml LNT) treatments were able to decrease the $OD_{540}$ value compared

with the cells in the control group. Treatment of HepG2 cells with a combination of 20 $\mu$g/ml oridonin and 200 $\mu$g/ml LNT increased the growth inhibitory rate compared with treatment with 200 $\mu$g/ml LNT alone (Table I).

*DNA content of sub-G1 HepG2 cells*. Following treatment of HepG2 cancer cells with LNT, the percentage of apoptotic cells (percentage of sub-G1DNA content) was increased compared with the control cells (2.3±0.4%; Fig. 3). The percentage of apoptotic HepG2 cells treated with a high concentration of LNT (LNT-H, 200 $\mu$g/ml) was 28.1±1.9%, and the percentage of cells treated with a low concentration of LNT (LNT-L, 100 $\mu$g/ml) was 16.8±1.8%. A nontoxic concentration (20 $\mu$g/ml) of oridonin was able to increase the percentage of apoptoticHepG2 cells that were also treated with LNT-H (oridonin + LNT-H vs. LNT-H, 43.7±2.8 vs. 28.1±1.9%; Fig. 3).

*mRNA and protein expression of caspase-3 and -9 in HepG2 cells*. The highest levels of caspase-3 and -9 mRNA expression were observed in HepG2 cells treated with oridonin and LNT-H, with a 3.10 and 2.51-fold increase compared with the control, respectively (Fig. 4). The oridonin + LNT-H treated HepG2 cells also exhibited higher caspase-3 and caspase-9 protein expression compared with the cells in the other groups (control, LNT-L and LNT-H).

*Gene and protein expression of Bax and Bcl-2 in HepG2 cells*. The levels of Bax mRNA expression in cells treated with oridonin + LNT-H, LNT-H and LNT-L demonstrated a3.71-, 1.70- and 2.42-fold increase compared with the control (Fig. 5). The levels of Bcl-2 mRNA expression in cells treated with oridonin + LNT-H, LNT-H and LNT-L demonstrated a0.21-, 0.48- and 0.76-fold decrease compared with the control. The highest protein expression of Bax was observed in the oridonin + LNT-H treatment group, and the lowest Bcl-2 protein expression was also observed in the oridonin + LNT-H group (Fig. 5).

*mRNA and protein expression of p53 and p21 in HepG2 cells*. The highest levels of p53 and p21 mRNA and protein expression were observed in the oridonin + LNT-H group. The levels of p53 and p21 were observed to be higher in the oridonin + LNT-H group compared with the LNT-L group (Fig. 6A and B). The levels of p53 and p21 mRNA expression in the oridonin + LNT-H group were 2.37 and 2.25 times higher compared with the control, respectively (Fig. 6A).

*mRNA and protein expression of NF-κB and IκB-α in HepG2 cells*. Treatment of cells with oridonin and LNT-H was able to reduce NF-κB expression and increase IκB-α expression compared with the control (Fig. 7). The levels of NF-κB mRNA was decreased 0.39-fold and the levels of IκB-α mRNA were increased 3.72-fold in cells treated with oridonin and LNT-H, compared with control cells (Fig. 7A). There was a similar trend in the expression of NF-κB and IκB-α proteins as the mRNA expression of these proteins (Fig. 7B).

## Discussion

Liver cancer cell apoptosis has an important role in the development of liver cancer. It is now known that there are a variety

Table I. Growth inhibition of HepG2 human hepatoblastoma cells by oridonin and lentinan as assessed by MTT assay.

| Treatment | OD$_{540}$ value | Inhibitory rate (%) |
|---|---|---|
| Oridonin + LNT-H | 0.076±0.006[a] | 84.3±2.5[a] |
| Control | 0.484±0.005[a] | - |
| LNT-L | 0.384±0.010[a] | 20.7±1.9[a] |
| LNT-H | 0.219±0.011[a] | 54.8±2.2[a] |

[a]P<0.05 vs. control, according to Duncan's multiple range test. Oridonin + LNT-H, 20 $\mu$g/ml oridonin + 200 $\mu$g/ml lentinan; LNT-L, 100 $\mu$g/ml lentinan; LNT-H, 200 $\mu$g/ml lentinan; OD, optical density.
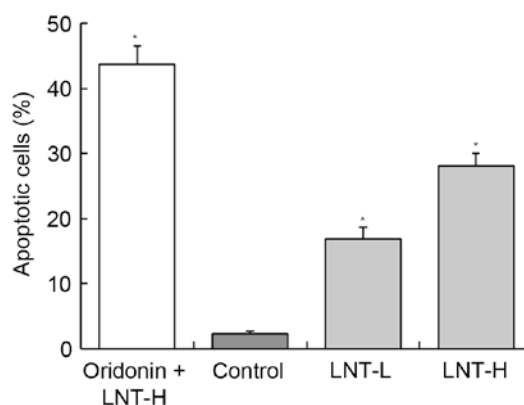


Figure 3. Flow cytometric analysis of the percentage of apoptotic HepG2 human hepatoblastoma cells. The DNA content of the sub-G1 phase was evaluated. [a-d]*Mean values with different letters over the bars are significantly different (P<0.05) according to Duncan's multiple range test. Oridonin + LNT-H, 20 $\mu$g/ml oridonin + 200 $\mu$g/ml lentinan; LNT-L, 100 $\mu$g/ml lentinan; LNT-H, 200 $\mu$g/ml; LNT, lentinan.

of cell signals mediated by receptors involved at the initiation of liver cancer cell apoptosis. There are also a number of proteases involved in apoptosis signal conduction as well as multiple genes involved in the regulation of liver cancer cell apoptosis. In previous years, it has been demonstrated that caspase-3 protease contributes a major function in apoptosis signaling transduction (15,16). The mechanism of caspase-3 protease activation is highly complex and regulated by various factors, thus there are different activation pathways. The activation of caspase-3 protease activation is closely associated with liver cancer cell apoptosis. Among the different proteases, caspase-3 has a key role in apoptosis; it is the core protease that causes caspase cascade reactions leading to apoptosis. Caspase-3 is activated in apoptosis of liver cancer cell and is induced by a variety of factors (17,18). Therefore, by inhibiting the activation and activity of caspase-3, this may inhibit the apoptosis of liver cancer cells (18).

Studies have reported have reported that Bcl-2 family members exert an important regulatory role in the process of caspase-3 activation (18,19). Anti-apoptotic member of Bcl-2 family, Bcl-xL inhibits oligomers of apoptotic protease-activating factor 1 (Apaf-1), which results in the loss-of-function of Apaf-1 molecules and inhibits Apaf-1-dependent activation of caspase-9 (20). Anti-apoptotic
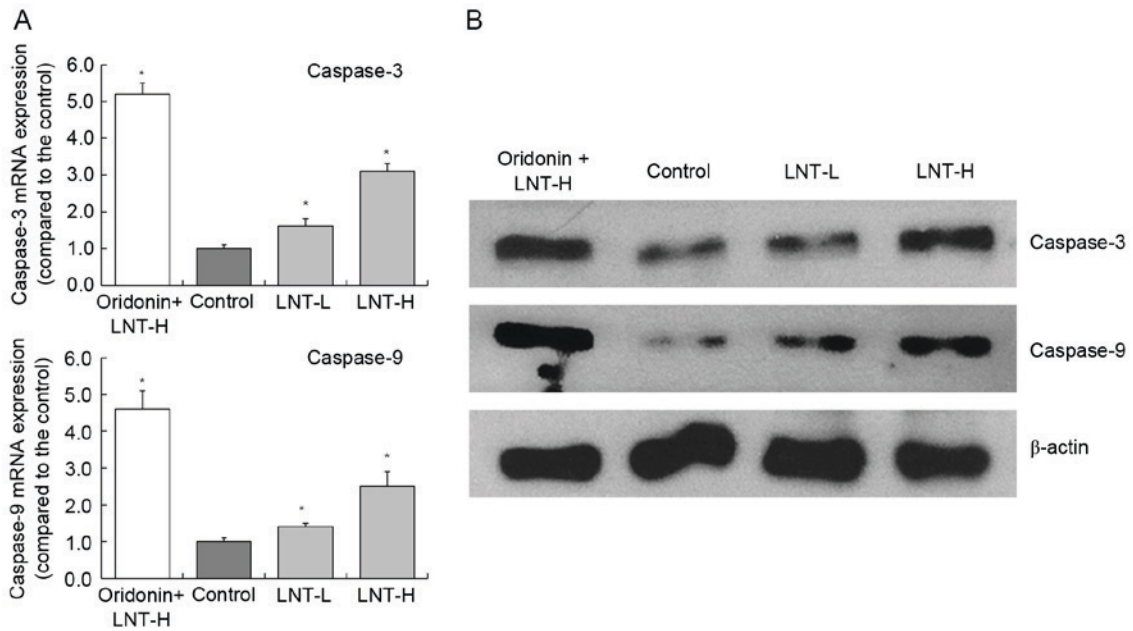
Figure 4. Expression of caspase-3 and caspase-9 in HepG2 human hepatoblastoma cells by reverse transcription-quantitative polymerase chain reaction and western blot analysis. (A) mRNA and (B) protein expression. *P<0.05 vs. control. Oridonin + LNT-H, 20 µg/ml oridonin + 200 µg/ml lentinan; LNT-L, 100 µg/ml lentinan; LNT-H, 200 µg/ml lentinan; LNT, lentinan.
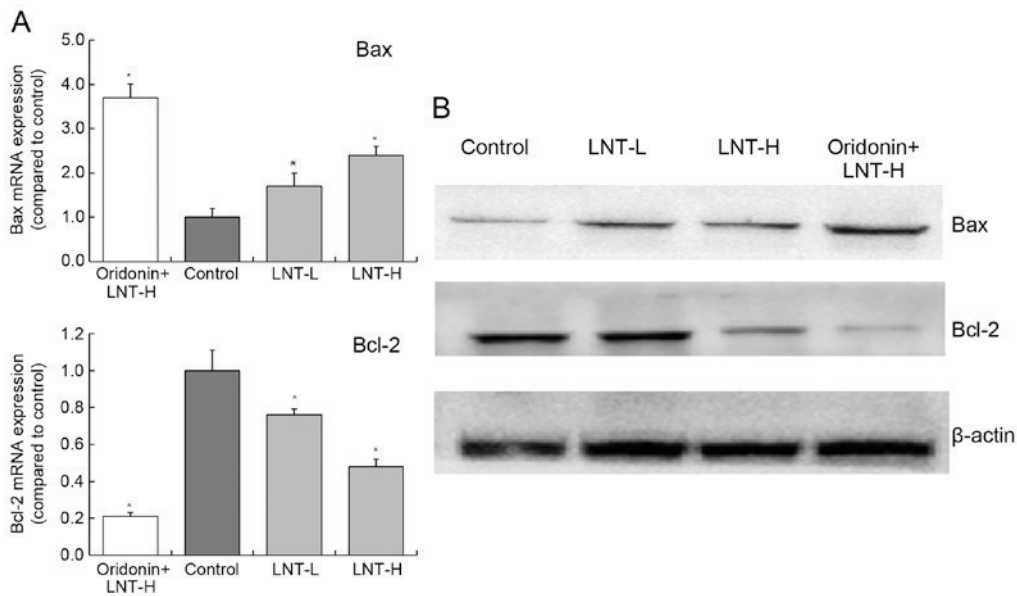


Figure 5. Expression of Bax and Bcl-2 in HepG2 human hepatoblastoma cells by (A) reverse transcription-quantitative polymerase chain reaction and (B) western blot analysis. *P<0.05 vs. control. Oridonin + LNT-H, 20 µg/ml oridonin + 200 µg/ml lentinan; LNT-L, 100 µg/ml lentinan; LNT-H, 200 µg/ml lentinan; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-like protein 4; LNT, lentinan.

members of the Bcl-2 family, which is primarily present in the outer membrane of the mitochondria, are able to prevent the release of cytochrome c from the mitochondria, which therefore inhibits the activation of pro-caspase-9 (21).

All pro-apoptosis Bcl-2 family members can form miscellaneous dimers with Bcl-2, Bcl-xL, A1 and Mcl-1 in the BH3 domain, which demonstrates that pro-apoptosis Bcl-2 family members, at least in part, function by interacting with anti-apoptosis Bcl-2 family members. Pro-apoptosis Bcl-2 family members are also able to induce the activation of caspases (22). Studies have also demonstrated that p53 is able

to cause apoptosis through the activation of caspases (23,24). Fuchs et al (25) reported that fas-deficient cells, which induced the expression of wild type p53, are able to activatecaspase-3 expression and characteristic changes in apoptosis, indicating that p53-dependent apoptosis can directly activate caspases, dependent on the involvement of Fas (25). Apoptosis inhibitory factor, Bcl-2, regulates apoptosis by forming dimer by itself or different dimers with Bax protein. An increase in theBcl-2/Bax ratio results in inhibition of apoptosis, yet if the ratio decreases apoptosis is promoted. The mechanism of action of Bcl-xL is similar to that of Bcl-2 (26). Silencing Bcl-2
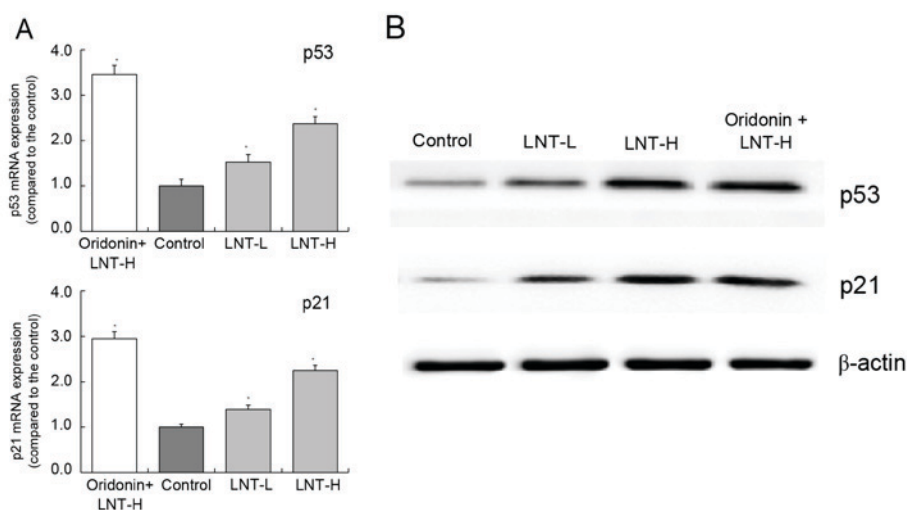
Figure 6. Expression of p53 and p21 in HepG2 human hepatoblastoma cells by (A) reverse transcription-quantitative polymerase chain reaction and (B) western blot analysis. $^{*}P<0.05$ vs. control. Oridonin + LNT-H, 20 $\mu$g/ml oridonin + 200 $\mu$g/ml lentinan; LNT-L, 100 $\mu$g/ml lentinan; LNT-H, 200 $\mu$g/ml lentinan; LNT, lentinan.
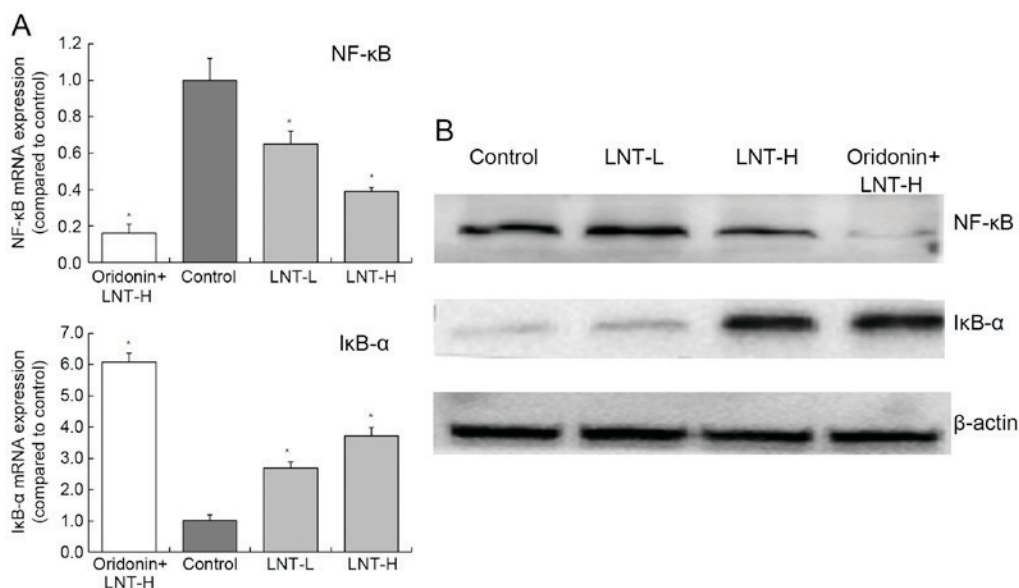


Figure 7. Expression of NF-κB and IκB-α in HepG2 human hepatoblastoma cells by (A) reverse transcription-quantitative polymerase chain reaction and (B) western blot analysis. $^{*}P<0.05$ vs. control. Oridonin + LNT-H, 20 $\mu$g/ml oridonin + 200 $\mu$g/ml lentinan; LNT-L, 100 $\mu$g/ml lentinan; LNT-H, 200 $\mu$g/ml lentinan; LNT, lentinan; NF-κB, nuclear factor κB; IκB-α, nuclear factor κB inhibitor α.

gene is also able to induce the activation of p53-dependent apoptotic signaling pathway. In p53 wild-type cancer cells, following the activation of p53, Bax expression increases if the concentration of ultraviolet radiation treatment increases. However, the expression of Bcl-2 and Bcl-xL decreases with the increase of inhibitor concentration. These findings indicated that in the process of killing cancer cells by inhibitors, p53 induces apoptosis primarily through the Bax/Bcl-2 and Bax/Bcl-xL signaling pathways (27). Another study has indicated that in cancer cells, ultraviolet radiation may change the expression of p21 through p53, therefore inducing cell cycle arrest. The increase in the expression of p21 and p53 is one of the markers that indicate that ultraviolet radiation is able to induce cancer cell apoptosis (28).

NF-κB is a type of nuclear transcription regulatory factor, which is present in the majority of cells. When the cell is not stimulated, NF-κB and its inhibitor IκB exist in cytoplasm in an activated form (29). However, when cells are stimulated by cellular damage or viruses, IκB is phosphorylated and degraded. This results in the translocation of NF-κB to the nucleus and consequently activation of NF-κB. Following activation, NF-κB can promote transcription of cytokines, chemokines and adhesion factors (30). In previous years, numerous studies have revealed that NF-κB can control proliferation, regulate cell cycle and apoptosis, affect differentiation, promote tumor metastasis and have a close association with the occurrence and development of tumors (30,31).

In the present study, MTT, flow cytometry, RT-qPCR and western blot analysis were performed. Treatment with LNT induced a decrease in cell viability in HepG2 cancer cells in a dose-dependent manner, and oridonin treatment promoted the anticancer effects of LNT *in vitro*. The results from the present study provide evidence that oridonin may be used to sensitize cells to LNT *in vitro*.

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