

Original Article

Resveratrol raises *in vitro* anticancer effects of lentinan in SW579 human thyroid squamous cell carcinoma

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Abstract: The aim of present study was to determine the *in vitro* Res (resveratrol) anticancer raising effects of LNT (lentinan) in SW579 human thyroid squamous cell carcinoma. By MTT assay, the no toxicity concentration of Res (10 µg/mL) in Nthy-ori 3-1 normal cells or SW579 cancer cells and no toxicity concentration of LNT (100 and 200 µg/mL) in Nthy-ori 3-1 normal cells were found. LNT-L (100 µg/mL) and LNT-H (200 µg/mL) showed the strong growth inhibitory effects in SW579 cells, and Res could raise growth inhibitory effects both of LNT-L and LNT-H treatment. By flow cytometry assay, the Res + LNT-H (10 µg/mL Res and 200 µg/mL LNT) treat SW579 cells had the most apoptosis cells, and after additional treated with Res, the apoptosis cells would be increased. By RT-PCR, the results showed that LNT could increase caspase-3, caspase-8, caspase-9, Bax, p53, p21, IκB-α, Fas, FasL, TIMP-1, TIMP-2 and decrease Bcl-2, Bcl-xL, NF-κB, EGF, EGFR, VEGF, Fit-1, MMP-2, MMP-9 mRNA expressions. The no effect low concentration of Res could raise the *in vitro* anticancer effects of LNT in SW579 cells, Res is a good sensitizing agent for LNT.

Keywords: Resveratrol, apoptosis, lentinan, SW579 human thyroid squamous cell carcinoma, cancer

Introduction

With the further development of molecular biology, people have gradually realized that polysaccharide has very important biological functions. Polysaccharides are closely related to the regulation of immune functions, the recognition among cells, transcellular transport, the diagnosis and treatment of cancer [1]. In recent years, researchers have found that the carbohydrate chain of polysaccharide can control the division and differentiation of cells, and regulate the growth and aging of cells [2]. Lentinane is one kind of fungi polysaccharide. As early as the 1960s, lentinan, as a high-quality immune promoter, was received wide attention and given a lot of researches. Researches have shown that lentinan has such functions as immunomodulation, antitumor, antiviral and anti-infection, etc [3-5].

The immunoregulatory activity of lentinan is vital for it to fight against cancer. Studies have found that the reason why lentinan can fight

against cancer cells and regulate immunity is because it can activate many immune cells such as T cells, natural killer cells and macrophages to increase immune cells' phagocytosis, so as to kill cancer cells in human's body, it can also kill migrated cancer cells. Lentinan can also regulate immune cells to release cell messengers such as co-stimulating factors [6]. Hou et al. [7] found lentinan can stimulate immune system to inhibit and antagonize the growth of cancer cells by increasing the amount of nitric oxide and other chemical messengers. In addition, lentinan can also enhance immune response of cancer cells in the human body, to combat against cancer by activating the specific reaction of cancers.

Resveratrol is a kind of polyphenol compound, mainly originating from peanut, grape (red wine), *Polygonum cuspidatum*, mulberry and other plants. Resveratrol is a kind of natural polyphenol with extensive biological functions, and a cancer chemopreventive agent, which can also reduce platelet aggregation, prevent

against and treat atherosclerosis, cardiovascular and cerebrovascular diseases [8]. In-depth research had found that resveratrol can change cell cycle, and influence the expression of apoptosis-related genes [9]. Fang et al. found that resveratrol can enhance the sensitizing effect of prostate cancer cell line PC-3 *in vitro* [10].

Cancer inhibitors exist in various plants, and have very good effect on human cancer prevention. These cancer inhibitors which come from natural plants are safe with low toxicity, and can reduce the pain of patients in treatment [11]. But the activity of many cancer inhibitors existing in natural plants is lower than that of synthetic drugs, and the combination of different natural cancer inhibiting substances can improve the treatment effect of cancer, so finding out a reasonable combination becomes the most important thing to improve the anti-cancer effect of natural substances. This study aims at finding out a new anti-cancer substance combination based on the researches that resveratrol can enhance anti-cancer treatment effect of lentinan *in vitro*, and verifying the treatment effect of the combined substances through the researches on the effect of combined resveratrol and lentinan on the inhibition of the growth of thyroid cancer cells, apoptosis induction and the conditions of cancer related genes *in vitro*, so as to provide theoretical basis for further animal experiment and clinical application.

Materials and methods

Cell line

Nthy-ori 3-1 normal human thyroid follicular epithelial cell was obtained from Health Protection Agency Culture Collections (HPACC; Porton Down, Salisbury, Wiltshire, UK) and SW579 human thyroid squamous cell carcinoma was obtained from Conservation Genetics CAS Kunming Cell Bank (Kunming, Guangxi, China).

MTT assay

Inoculate SW579 cancer cells in DMEM culture solutions containing 10% of inactivated calf serum, place it into incubator under fully humidification at 37°C with 5% of CO₂, replace culture medium 2 to 3 times a week, and secondarily culture cells every 6 to 7 d. Then in ulate cul-

tured cancer cells onto 96-well culture plate by 1×10^4 /mL, with 180 μ L per well. Then cultivate it at 37°C with 5% of CO₂ under fully humidification for 24 h. when cells adhere to walls, add 20 μ L of resveratrol or lentinan solution into each well, after 48 hours of cultivating, add 20 μ L of MTT reagent in each well (with a concentration of 5 mg/mL) to cultivate it for 4 hours. Afterwards, drain away the supernate in the well after cultivating ends, shake culture plate after adding 150 μ L of DMSO into each well for 30 minutes, at last, measure the diameter of each well under 540 nm wavelength by standard microplate reader and calculate cellular proliferation inhibition rates [12].

Flow cytometry

Use dbcAMP to treat cancer cells cultured for 24 h, add 0.25% of pancreatic enzymes to digest and detach cells, when cells fall off, add in culture medium containing serum to neutralize the effect of pancreatic enzyme, then centrifuge it for 5 min, drain away the supernate and collect sediments, which will be cleaned with PBS, and fixed by 75% of ethanol later. Adjust the concentration of the cancer cells to 5×10^5 /mL, after washing it with PBS for three times, add in PBS, RNase and Triton which all contain EB (ethidium bromide), incubate them at 4°C in dark for 30 minutes, then test and detect them by flow cytometry [13].

RT-PCR assay

Prepare total RNAs of cancer cells under the same conditions by using RNazol reagents. After the quantitative separation of RNA, use oligodT as primer to prepare ss cDNA by AMV reverse transcriptase and then use cDNA as template to amplify Bax gene by RT-PCR method with housekeeping gene GAPDH as internal reference. After the experiment ends, use agarose gel (containing 1% ethidium bromide) to check the PCR amplification products [14].

Statistical analysis

The *in vitro* experiments were presented as mean \pm standard deviation (SD). Differences between the mean values for individual groups were assessed with one-way analysis of variance (ANOVA) with Duncan's multiple range test using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) [14].

Resveratrol raises anticancer effects of lentinan

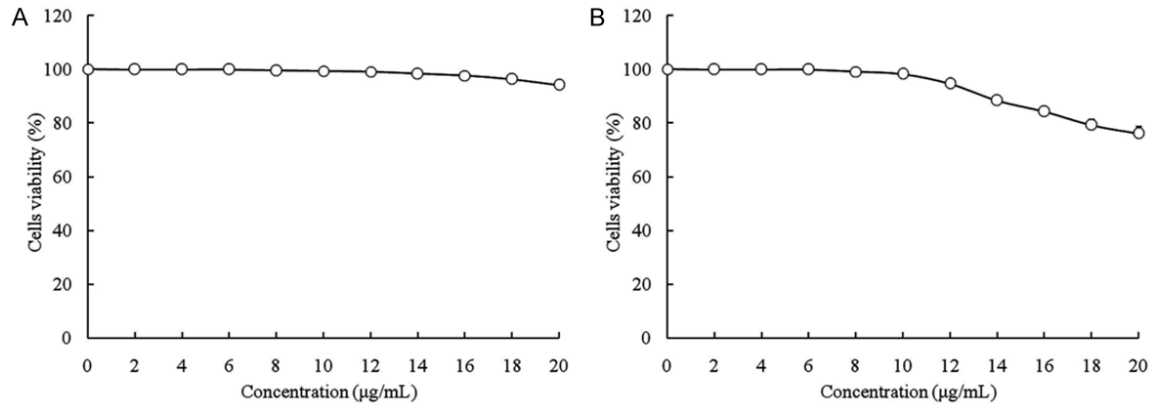


Figure 1. Effect of resveratrol on the growth of Nthy-ori 3-1 normal human thyroid follicular epithelial cell (A) and SW579 human thyroid squamous cell carcinoma (B) by MTT assay.

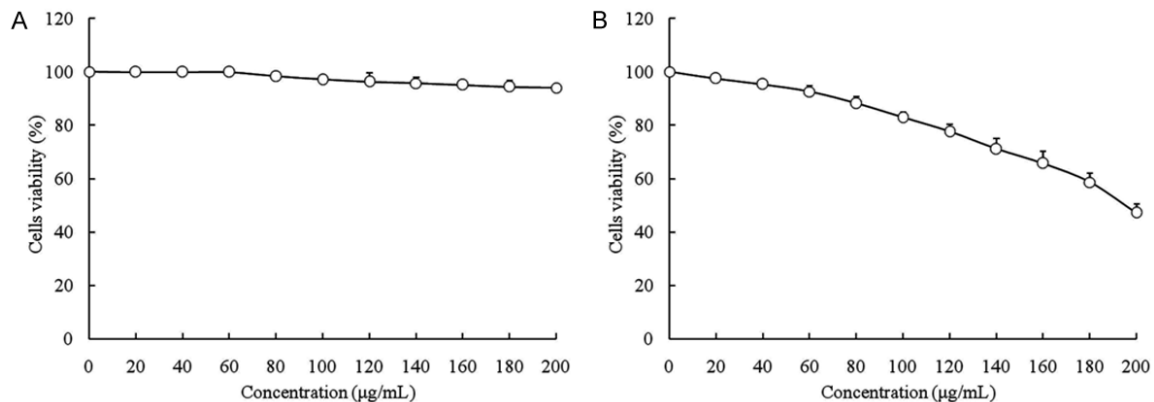


Figure 2. Effect of lentinan on the growth of Nthy-ori 3-1 normal human thyroid follicular epithelial cell (A) and SW579 human thyroid squamous cell carcinoma (B) by MTT assay.

Results

The growth of resveratrol and lentinan in Nthy-ori 3-1 and SW579 cells

The growth of resveratrol (Res) and lentinan (LNT) in Nthy-ori 3-1 and SW579 cells were determined by MTT assay (**Figures 1** and **2**). At the concentration of 0-10 μl/mL, Res could not reduce the growth in Nthy-ori 3-1 or SW579 cells, these concentration hadn't toxicity in normal or cancer cells (**Figure 1**). LNT could reduce the growth in SW579 cancer cells depend on the concentration of 0-200 μl/mL, but LNT could not reduce the growth in Nthy-ori 3-1 normal cells in these concentrations, 0-200 μl/mL of LNT had only effects in cancer cells. Base on this results, 10 μl/mL of Res was chose as enhancement concentration for increasing LNT anticancer effect determination, and 100, 200

μl/mL were chose for LNT anticancer effects checking (**Figure 2**).

By the further MTT results, no sample treated SW579 cancer cells showed the OD₅₄₀ value at 0.462 (**Table 1**), LNT-L (100 μg/mL lentinan), Res + LNT-L (10 μg/mL resveratrol + 100 μg/mL lentinan), LNT-H (200 μg/mL lentinan) and Res + LNT-H (10 μg/mL resveratrol + 200 μg/mL lentinan) treatments could reduce the OD₅₄₀ value compared to the control cells. Res (10 μg/mL) additional treatment could raise the growth inhibitory rate compared to the same concentration of LNT.

DNA content of sub-G1 SW579 cells

By the flow cytometer assay, the results showed that control cells has the fewer apoptotic SW579 cancer cells (3.1±0.6% DNA content of

Table 1. Growth inhibition of SW579 human thyroid squamous cell carcinoma by resveratrol and lentinan by an MTT assay

Treatment	OD ₅₄₀ value	Inhibitory rate (%)
Control (untreated)	0.462±0.004 ^a	/
LNT-L	0.383±0.010 ^b	17.1±1.9 ^D
Res + LNT-L	0.302±0.013 ^c	34.6±2.4 ^C
LNT-H	0.218±0.009 ^d	52.8±3.2 ^B
Res + LNT-H	0.103±0.008 ^e	77.7±2.7 ^A

^{a-e, A-D}Mean values with different letters in the same column are significantly different ($P<0.05$) according to Duncan's multiple-range test. LNT-L: 100 µg/mL lentinan; Res + LNT-L: 10 µg/mL resveratrol + 100 µg/mL lentinan; LNT-H: 200 µg/mL lentinan; Res + LNT-H: 10 µg/mL resveratrol + 200 µg/mL lentinan.

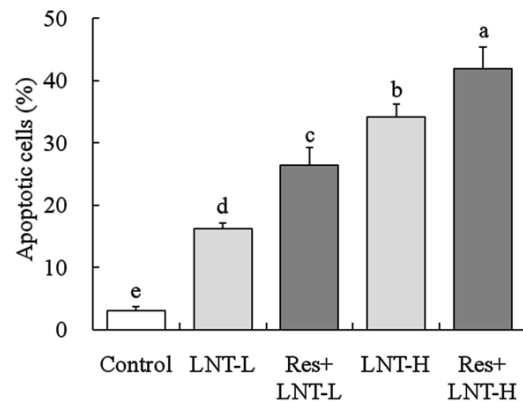


Figure 3. The apoptotic cells (DNA content of sub-G1) of SW579 human thyroid squamous cell carcinoma was evaluated using a flow cytometer. a-e. Mean values with different letters over the bars are significantly different ($P<0.05$) according to Duncan's multiple-range test. LNT-L: 100 µg/mL lentinan; Res + LNT-L: 10 µg/mL resveratrol + 100 µg/mL lentinan; LNT-H: 200 µg/mL lentinan; Res + LNT-H: 10 µg/mL resveratrol + 200 µg/mL lentinan.

sub-G1), 100 and 200 µg/mL LNT have more apoptotic SW579 cancer cells (16.3±0.9% and 34.2±2.1% DNA content of sub-G1), after additional treatment by 10 µg/mL Res, the apoptotic SW579 cancer cells of 100 and 200 µg/mL LNT were raised at 26.4±2.9% and 41.9±3.5% (Figure 3).

mRNA gene expression of caspases

The mRNA expression of caspase-3, caspase-8 and caspase-9 in SW579 cancer cells were determined by RT-PCR assay (Figure 4). The control cells had the weakest caspase-3, cas-

pase-8 and caspase-9 expressions, 100 (1.19, 1.60 and 1.32 folds over control) and 200 µg/mL (2.10, 1.98 and 2.27 folds over control) LNT treated cells showed the higher expression than control cells, 10 µg/mL Res additional treatment for 100 (1.73, 1.74 and 1.81 folds over control) and 200 µg/mL (2.39, 2.31 and 2.51 folds over control) LNT could increase the caspase-3, caspase-8 and caspase-9 expressions.

mRNA gene expression of Bax, Bcl-2 and Bcl-xL

The Bax mRNA expression of LNT-L, Res + LNT-L, LNT-H and Res + LNT-H treated SW579 cancer cells were 1.66, 2.47, 2.66 and 3.24 folds over control cells (Figure 5). Bcl-2 and Bcl-xL expression of LNT-L, Res + LNT-L, LNT-H and Res + LNT-H treatment were lower than no treatment (control), and Res + LNT-H showed the lowest expressions (0.29 and 0.03 folds over control).

mRNA gene expression of p53 and p21

The Res + LNT-H treated SW579 cancer cells had the strongest p53 and p21 expressions (3.78 and 3.33 folds over control), these expressions of Res additional treatment (Res + LNT-L and Res + LNT-H) were both higher than the same concentrations of LNT (LNT-L and LNT-H) (Figure 6).

mRNA gene expression of NF-κB and IκB-α

The mRNA gene expression of NF-κB in LNT-L, Res + LNT-L, LNT-H and Res + LNT-H treated SW579 cancer cells were 0.66, 0.58, 0.47 and 0.33 folds over control cells respectively (Figure 7). And IκB-α expression of in these group cells were 1.21, 1.68, 1.72 and 2.98 folds over control cells respectively.

mRNA gene expression of EGF and EGFR

The EGF and EGFR mRNA gene expression of Res + LNT-L (0.13 and 0.35 folds over control) and Res + LNT-H (0.02 and 0.09 folds over control) treated SW579 cancer cells were lower than LNT-L (0.18 and 0.65 folds over control) and LNT-H (0.06 and 0.22 folds over control) treated SW579 cells, and EGF and EGFR expression in group cells were all lower than control group (Figure 8).

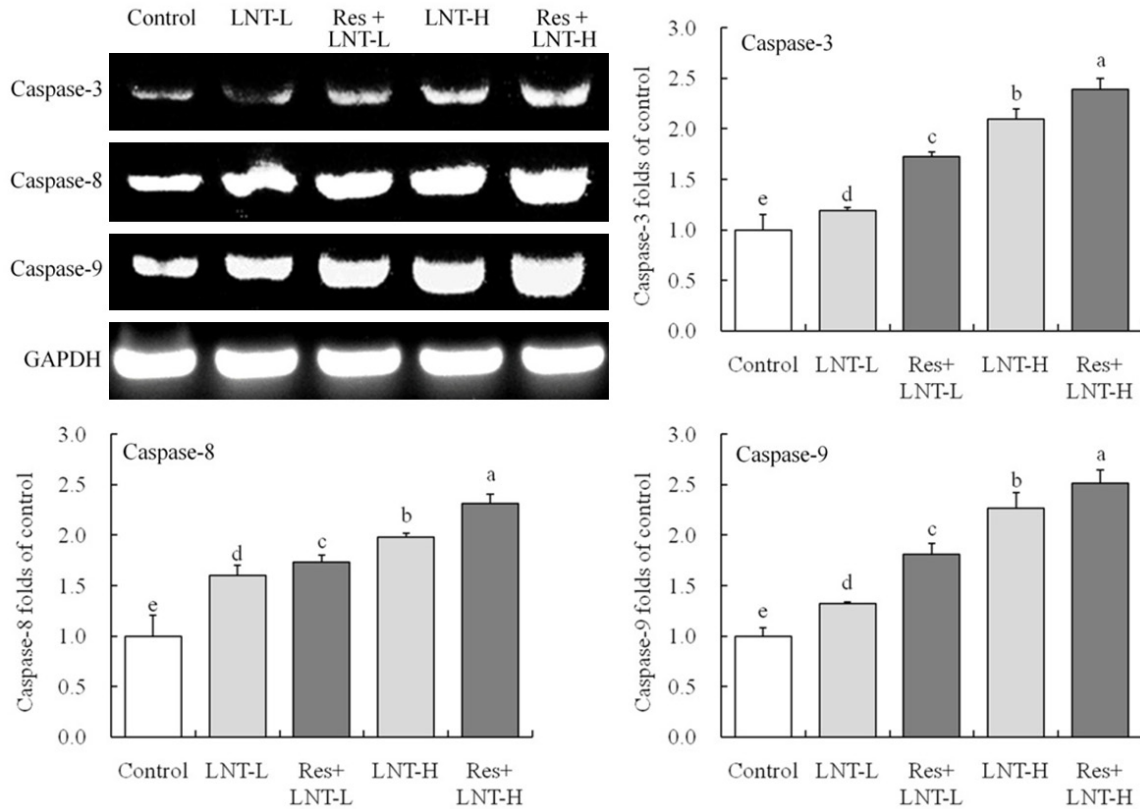


Figure 4. The mRNA expression of caspase-3, caspase-8 and caspase-9 in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g}/\text{mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g}/\text{mL}$ resveratrol + 100 $\mu\text{g}/\text{mL}$ lentinan; LNT-H: 200 $\mu\text{g}/\text{mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g}/\text{mL}$ resveratrol + 200 $\mu\text{g}/\text{mL}$ lentinan.

mRNA gene expression of VEGF and Fit-1

The Res + LNT-H treatment could reduce the VEGF and Fit-1 expression to 0.02 and 0.06 folds over control (**Figure 9**), these expressions were lower than other groups, and after additional treated with Res (10 $\mu\text{g}/\text{mL}$), both LNT-L and LNT-H treated cells had the lower VEGF and Fit-1 expression than only LNT-L and LNT-H treated cells.

mRNA gene expression of Fas and FasL

The Fas and FasL gene expressions in LNT-L (2.61 and 1.60 folds over control) and LNT-H (5.08 and 2.66 folds over control) treatment groups were stronger than control group (**Figure 10**). And Res + LNT-L (3.36 and 2.00 folds over control) and Res + LNT-H (5.40 and 3.16 folds over control) treatment could raise Fas and FasL expressions compared to LNT-L and LNT-H treatment.

mRNA gene expression of MMP-2 and MMP-9

After treated with LNT, the MMP-2 and MMP-9 were decreased compared to the control cells (**Figure 11**). And the Res could raise of MMP-2 and MMP-9 expression decreasing of LNT treatment, and Res + LNT-H showed the lowest MMP-2 (0.21 folds over control) and MMP-9 (0.40 folds over control) expression.

mRNA gene expression of TIMP-1 and TIMP-2

Res + LNT-H treated cells had the strongest TIMP-1 and TIMP-2 expressions (3.92 and 3.12 folds over control), and these expression in Res + LNT-L treated cells (2.21 and 1.87 folds over control) were stronger than only LNT-L treated cells (1.22 and 1.38 folds over control) and control cells (**Figure 12**).

Discussion

Cancer cell apoptosis plays an important role in the occurrence and development of cancer,

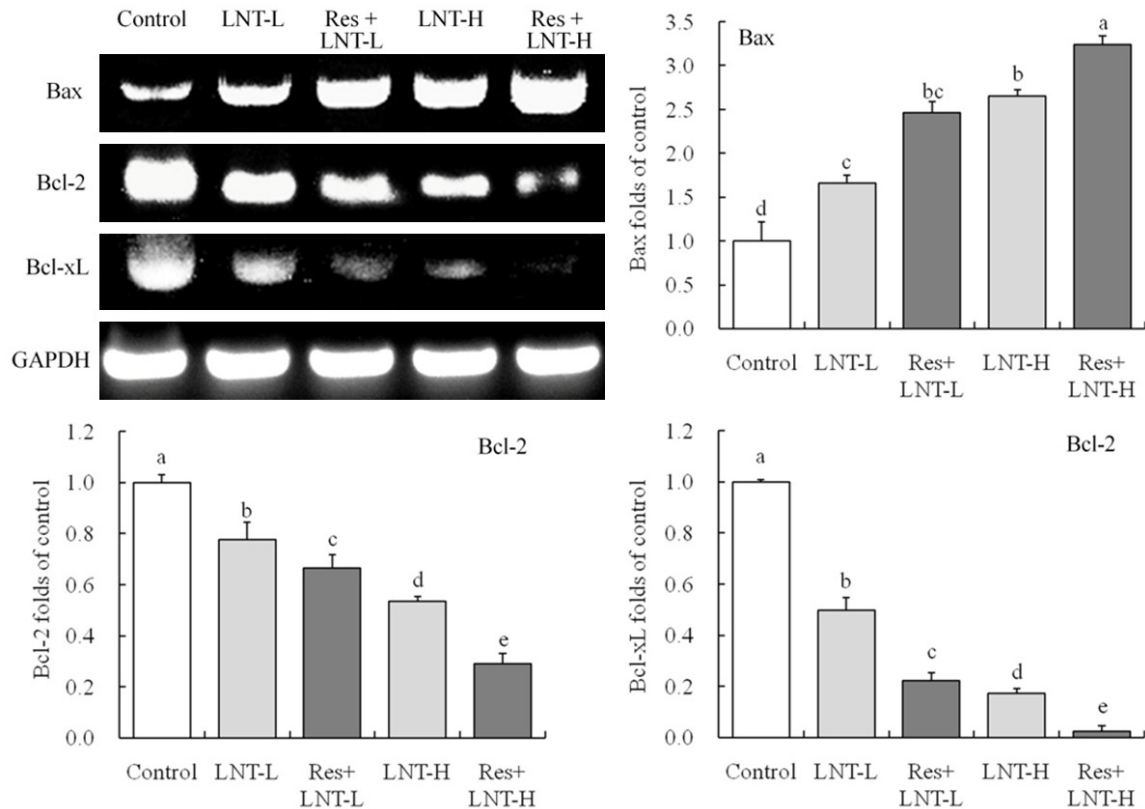


Figure 5. The mRNA expression of Bax, Bcl-2 and Bcl-xL in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g/mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g/mL}$ resveratrol + 100 $\mu\text{g/mL}$ lentinan; LNT-H: 200 $\mu\text{g/mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g/mL}$ resveratrol + 200 $\mu\text{g/mL}$ lentinan.

studies have found out a lot of receptor-mediated cell signal transduction get involved in the activation of cancer cells apoptosis, and many genes are related to the regulation of cancer cell apoptosis [15]. The activation of caspase-3 is closely related to cancer cell apoptosis. Existing evidences have suggested that protease is in close relation to cell apoptosis, which may be the cascading process of protease. Caspase-3 is the "core" protease of activating the cascade effect of apoptotic protease, playing a very important role in this process. Caspase-3 can activate apoptotic cancer cells, so inhibiting the activity of caspase-3 can prevent the apoptosis of liver cancer cells [16]. There are two caspase-3 activation dependent manners: caspase-8 or caspase-10 dependent manner, and caspase-10 dependent manner. The former can transmit apoptosis signal through the binding of death ligands such as Fas, TNFR1 and DR3 with death receptors; the latter can stimulate chondriosome to release cytochrome c by the use of chemical drugs and

the removal of survival factor, cytochrome c will bind to WD40 repeat protein of Apaf-1 when dATP or ATP appear, then Apaf-1 and procaspase-9 can bind together through their respective CARD [17].

Bcl-2 can inhibit cell apoptosis, and prolong the survival period of tumor cells, to cause cancer recur and metastasize [18]. Experimental study had found that Bcl-2 family members play an important regulating role in caspase-3 activation [19]. Bcl-xL, a member of Bcl-2 anti-apoptotic family, can inhibit the action of oligomers in Apa-1 molecules to make Apaf-1 molecular loss its function, thus inhibiting the activation of Apaf-1-dependent caspase-9. Anti-apoptotic Bcl-2 and Bax concentrated on the outer membrane of mitochondria can inhibit mitochondria to release cytochrome c, so as to restrain the activation of caspase-9 [20]. While all apoptosis-promoting Bcl-2 family members can form dipolymers through the reaction of BH3 domain protein with Bcl-2, Bcl-xL, A1 and Mcl-1, indicat-

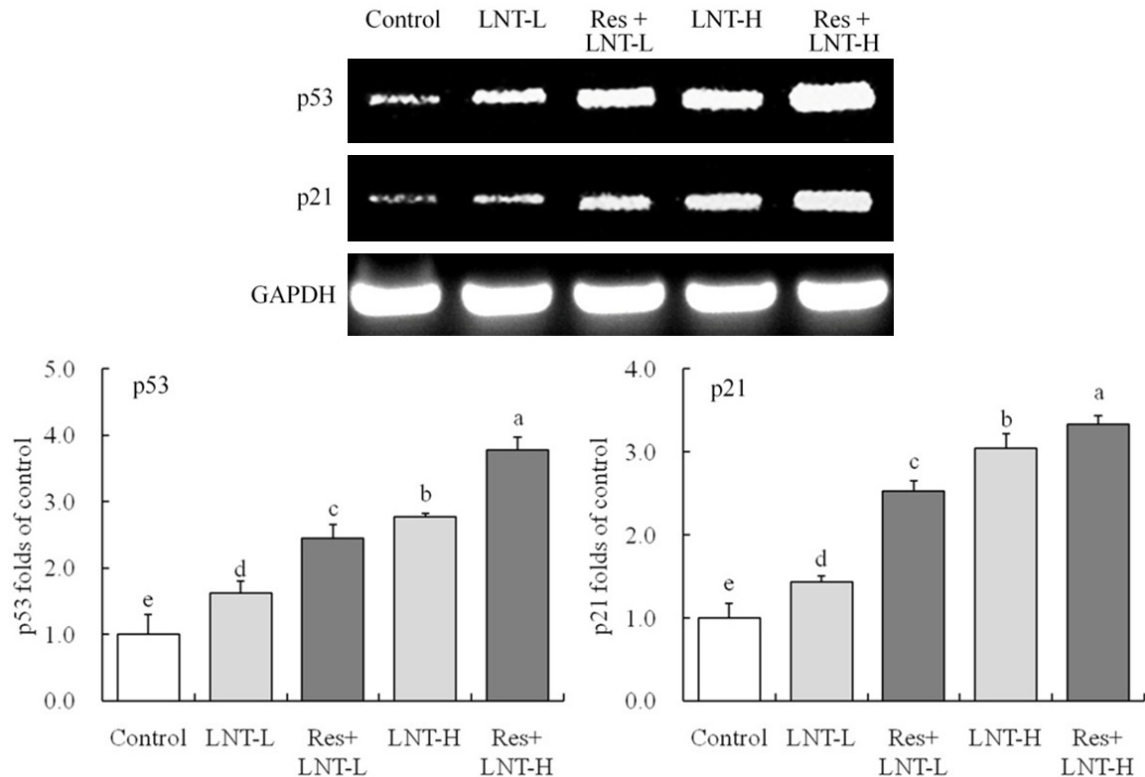


Figure 6. The mRNA expression of p53 and p21 in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g/mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g/mL}$ resveratrol + 100 $\mu\text{g/mL}$ lentinan; LNT-H: 200 $\mu\text{g/mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g/mL}$ resveratrol + 200 $\mu\text{g/mL}$ lentinan.

ing at least part of apoptosis-promoting Bcl-2 family react due to its resistance with anti-apoptotic Bcl-2 family. Therefore, Bcl-2 can induce the activation of caspases [21].

Study has shown that more than 50% of tumors can lead to p53 mutation, which may change the expression of its downstream genes, resulting in the changes in cell apoptosis and cell cycle arrest, inducing or promoting the occurrence and development of malignant tumor [22]. Study has found that p53 can cause apoptosis by activating caspases [23], Klostergaard et al. [24] found if the cell with Fas defect was given with wild-type p53, it can lead to the activation of caspase-3, and characteristic changes such as cell apoptosis. In the process of killing cancer cells, p53 mutation can induce cell apoptosis mainly through Bax/Bcl-2 protein and Bax/Bcl-xL protein [24]. Experimental results have shown that in cancer cells, active substances can change the expression of p21 due to p53 mutation, to induce cell cycle arrest, the enhanced expression of p21 and p53 is a

mark that active substances induce the apoptosis of cancer cells [25, 26].

NF- κ B is a kind of nuclear transcription regulatory factor, it exists in almost all cells. When not being stimulated, NF- κ B binds to its inhibitor I κ B in an inactivated form in cytoplasm, when cells get damaged, or stimulated by virus, I κ B will get phosphorylated and degraded, causing NF- κ B to move into nucleus rapidly and activate it, promoting the transcription of cytokines, chemokines and adhesion factors [27]. In recent years, studies have found that NF- κ B can control cell proliferation, regulate cell cycle and apoptosis, affect cell differentiation and promote tumor metastasis, it has close relationship with the occurrence and development of tumors [28, 29].

EGF is a kind of growth factor which can affect many reactions by combining with EGFR [30]. Study has shown that EGF and other growth factors can promote the proliferation of human cells. EGFR is a member of ErbB receptor family

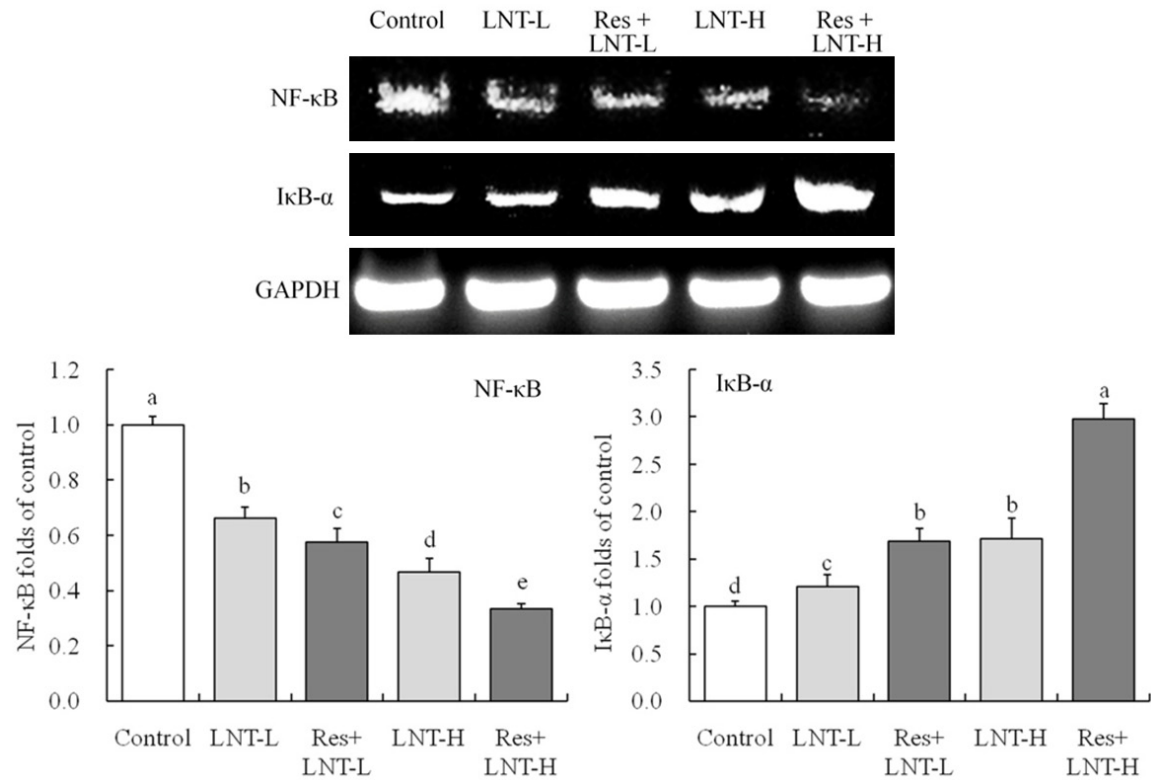


Figure 7. The mRNA expression of NF- κ B and I κ B- α in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 μ g/mL lentinan; Res + LNT-L: 10 μ g/mL resveratrol + 100 μ g/mL lentinan; LNT-H: 200 μ g/mL lentinan; Res + LNT-H: 10 μ g/mL resveratrol + 200 μ g/mL lentinan.

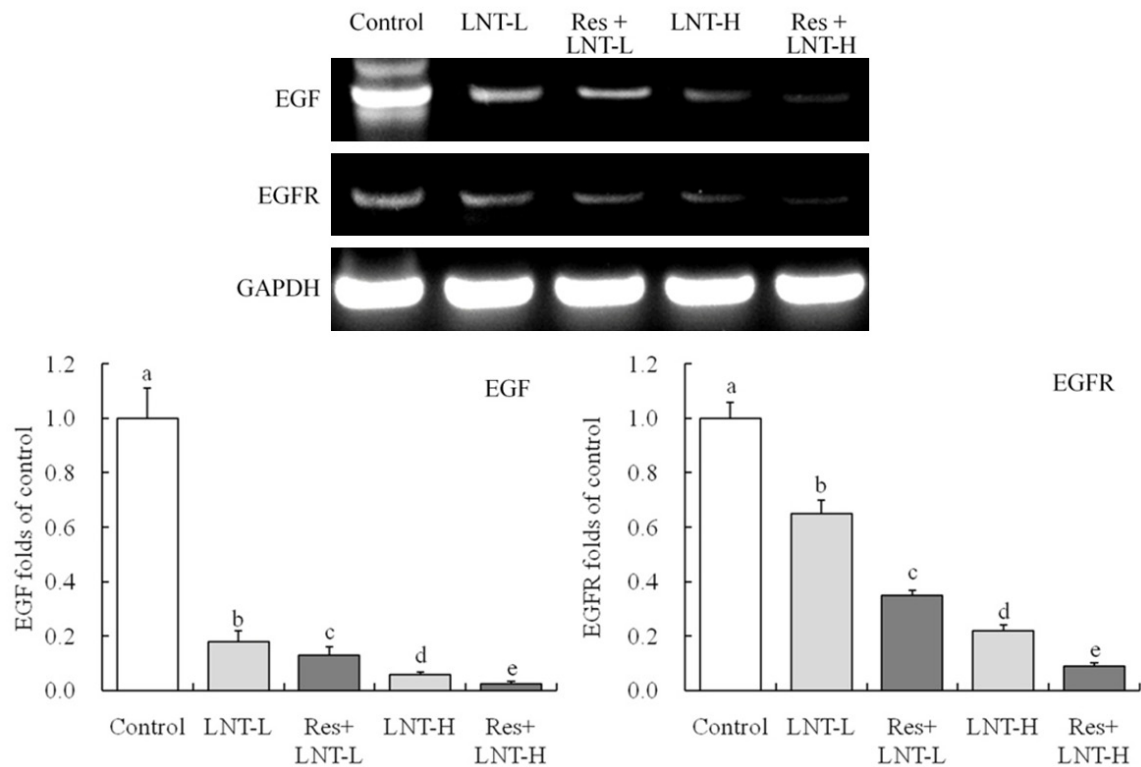


Figure 8. The mRNA expression of EGF and EGFR in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g/mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g/mL}$ resveratrol + 100 $\mu\text{g/mL}$ lentinan; LNT-H: 200 $\mu\text{g/mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g/mL}$ resveratrol + 200 $\mu\text{g/mL}$ lentinan.

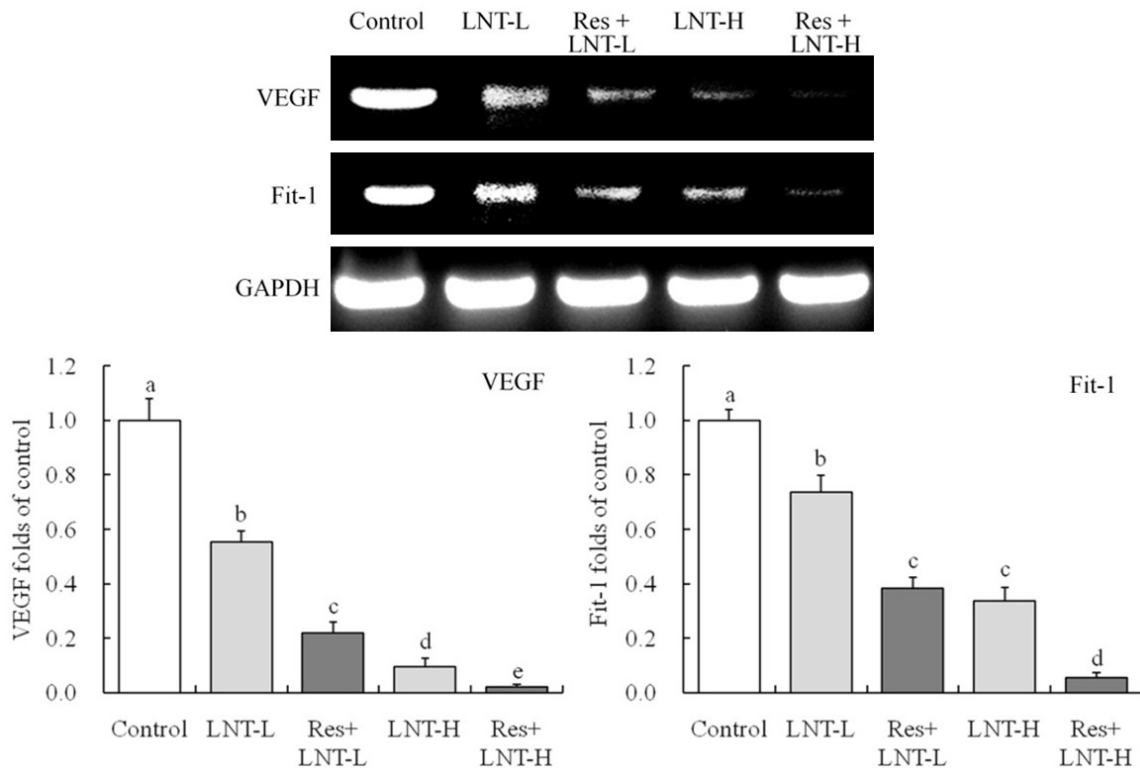


Figure 9. The mRNA expression of VEGF and Fit-1 in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g/mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g/mL}$ resveratrol + 100 $\mu\text{g/mL}$ lentinan; LNT-H: 200 $\mu\text{g/mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g/mL}$ resveratrol + 200 $\mu\text{g/mL}$ lentinan.

located on the surface of cells, involved in cells proliferation, growth, migration and infiltration, etc [31]. Study has found that EGFR can adjust EGF-mediated cancer cell proliferation through sialylation [32].

VEGF can promote the growth of tumor and angiogenesis, and provides a foundation for tumor metastasis, affecting the prognosis of patients with tumor. VEGF is the strongest vascular endothelial cell growth factor which can directly work on blood vessels, and specifically promote the division, proliferation and migration of endothelial tumor cells, playing an important role in the formation of tumor blood vessel, and it is also one of the key factors of promoting angiogenesis [33]. Fit-1 is the receptor of VEGF, it can bind to VEGF in high affinity. Fit-1 receptor deficient mice are mainly charac-

terized by vascular endothelial cell damage, the expression of Fit-1 is mainly related to the early-stage angiogenesis and wound healing of mouse embryos [34].

Fas is a common death receptor, if binding to FasL, it can be activated to transmit signal, leading to apoptosis, which is one of the major causes of cell apoptosis. When Fas binds to FasL, FADD domain protein in Fas intracellular domain can activate the recruitment response of caspase-8 and caspase-10, so as to activate downstream caspases protein, leading to the apoptosis which expresses Fas cells [35]. In addition, the metastasis of tumor is closely related to its resistance. Some literature report that the activation of Fas pathway can strengthen the resistance to apoptosis cells, which FasL plays a vital role [36].

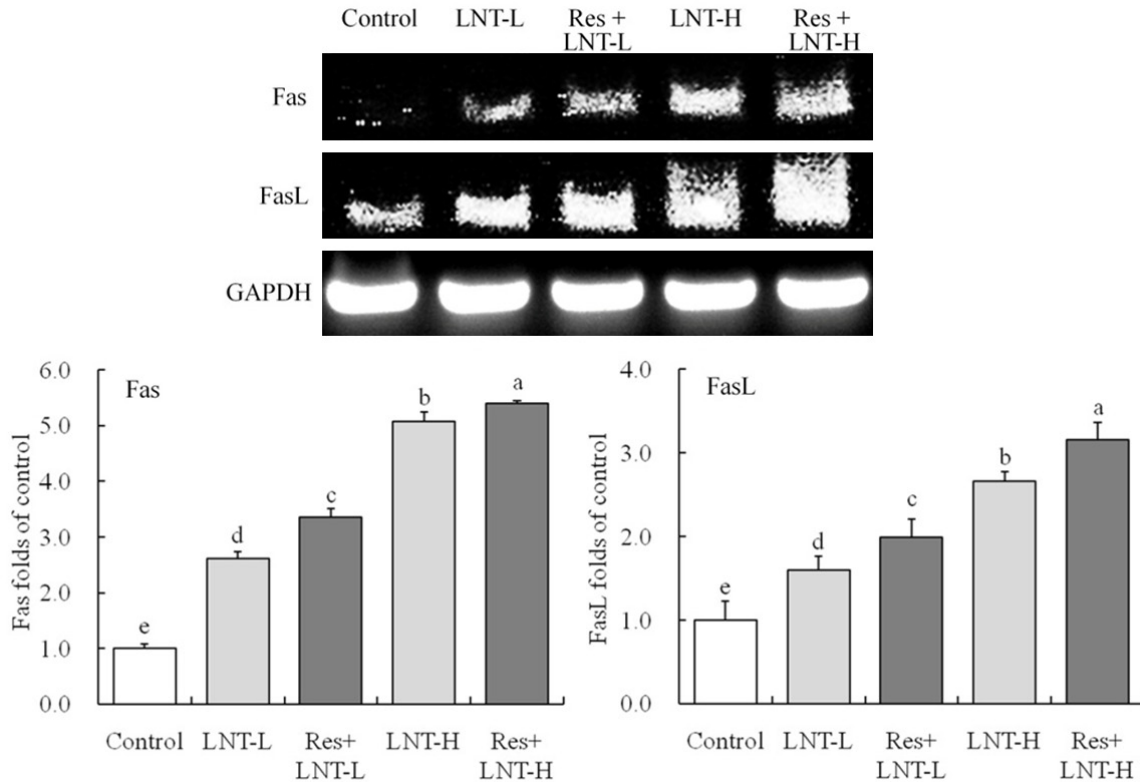


Figure 10. The mRNA expression of Fas and FasL in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g/mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g/mL}$ resveratrol + 100 $\mu\text{g/mL}$ lentinan; LNT-H: 200 $\mu\text{g/mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g/mL}$ resveratrol + 200 $\mu\text{g/mL}$ lentinan.

Malignant tumors are characterized by local invasion and distant metastasis, which are the main reasons that malignant tumor threaten patients' health and life. MMPs play an important role in the invasion and metastasis of tumor, it not only mediates tumor cells' degradation of extracellular matrix including the basement membrane, but controls the process of angiogenesis, affects the function of cell adhesion molecules and regulates the growth of tumor cells (in situ and ex situ), etc [37]. Study has shown that the expression of MMP-2 and MMP-9 is closely related to cancer angiogenesis; tumor cells which can secrete MMP-2 and MMP-9 have high invasion and metastases ability, drugs can also be used to inhibit the growth of tumor cells through lowering the activity of MMP-2 and MMP-9 [38]. In addition, ECM play a key role in local invasion and distant metastasis of cancer cells, the degradation of ECM is complex, involved a lot of factors, and MMPs and inhibitors play important functions, MMPs can degrade ECM, while TIMPs can

inhibit the degradation of ECM through lowering the activity of MMPs, to protect normal cells [39]. The formation of intravascular cavity depends on the balance of MMPs and TIMPs, the adding of exogenous inhibitors may break the balance of MMPs and TIMPs, inhibiting the process of angiogenesis, and the invasion and metastasis of tumor cells. TIMPs can inhibit tumor invasion and metastasis, therefore, it has been used in the research of tumor treatment [40].

In this study, after MTT, flow cytometry and RT-PCR assay determinations, LNT showed a good *in vitro* anticancer in SW579 human thyroid squamous cell carcinoma, and no toxicity concentration of Res could increase the anticancer effects of LNT. From these results, Res could be used as a sensitizing agent for LNT *in vitro*.

Disclosure of conflict of interest

None.

Resveratrol raises anticancer effects of lentinan

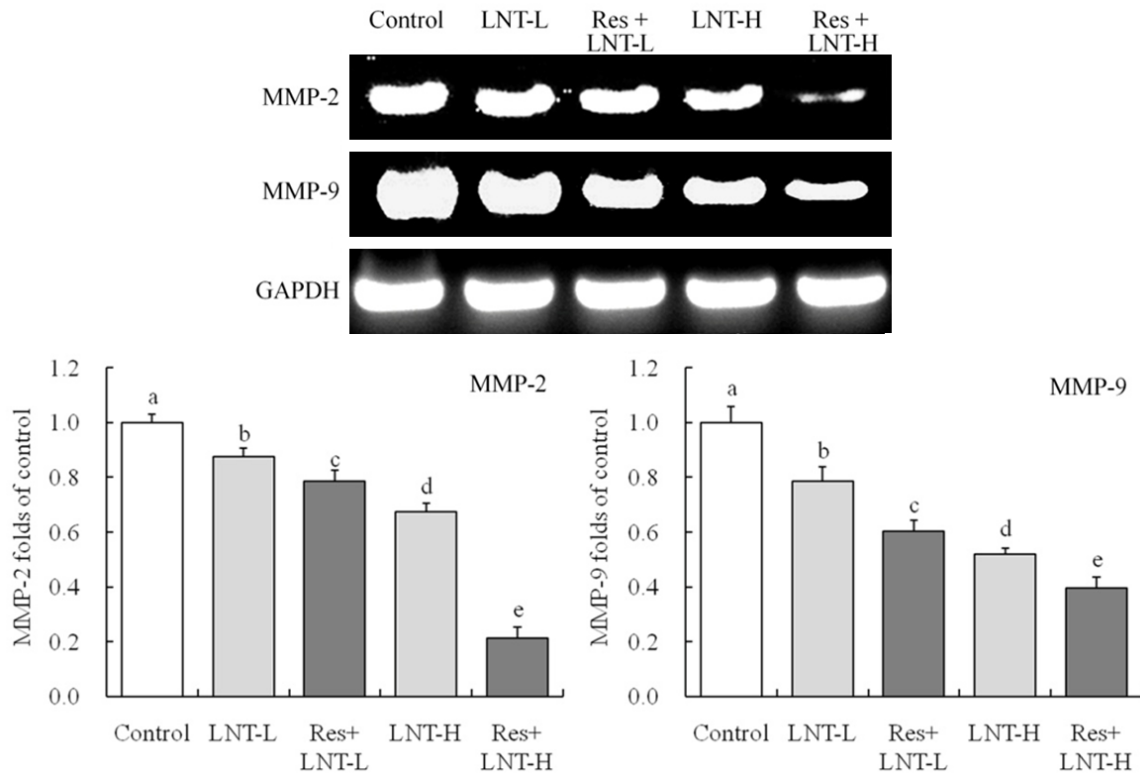


Figure 11. The mRNA expression of MMP-2 and MMP-9 in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g/mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g/mL}$ resveratrol + 100 $\mu\text{g/mL}$ lentinan; LNT-H: 200 $\mu\text{g/mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g/mL}$ resveratrol + 200 $\mu\text{g/mL}$ lentinan.

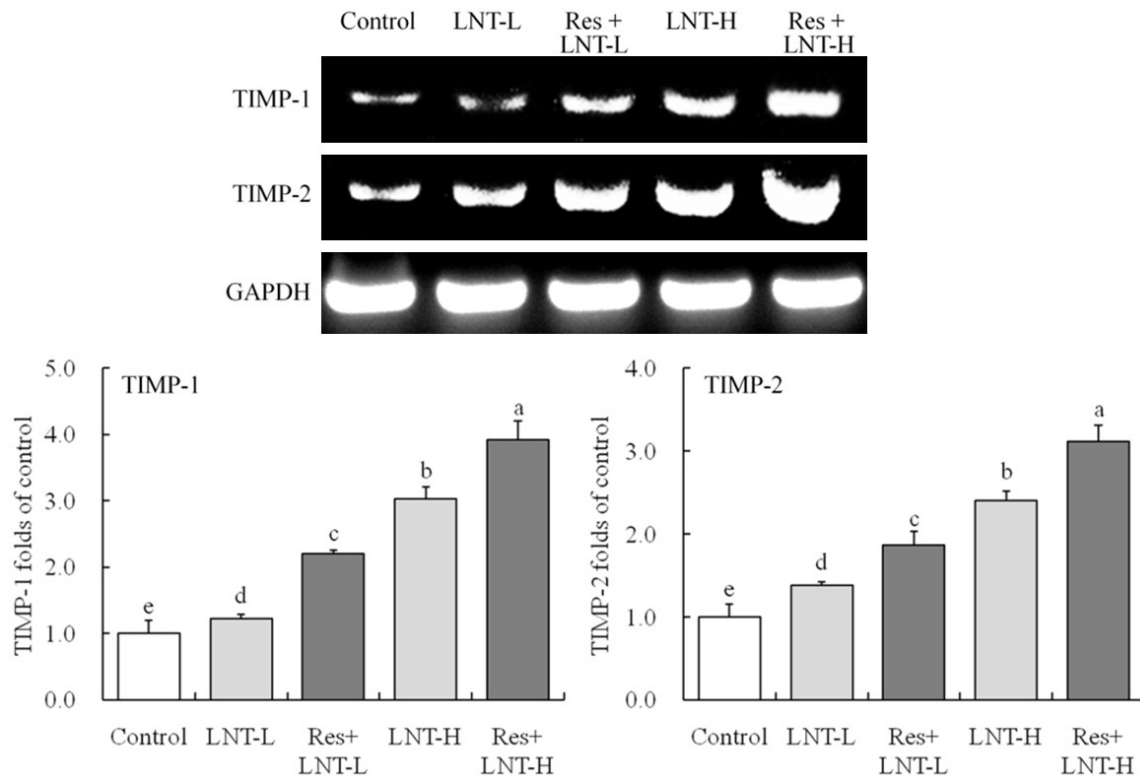


Figure 12. The mRNA expression of TIMP-1 and TIMP-2 in SW579 human thyroid squamous cell carcinoma by RT-PCR assay. a-e. Mean values with different letters over the bars are significantly different ($P < 0.05$) according to Duncan's multiple-range test. LNT-L: 100 $\mu\text{g/mL}$ lentinan; Res + LNT-L: 10 $\mu\text{g/mL}$ resveratrol + 100 $\mu\text{g/mL}$ lentinan; LNT-H: 200 $\mu\text{g/mL}$ lentinan; Res + LNT-H: 10 $\mu\text{g/mL}$ resveratrol + 200 $\mu\text{g/mL}$ lentinan.

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