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RESEARCH ARTICLE

Resveratrol inhibits human lung adenocarcinoma cell metastasis by suppressing heme oxygenase 1-mediated nuclear factor-κB pathway and subsequently downregulating expression of matrix metalloproteinases

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Resveratrol exhibits potential anti-carcinogenic activities. Heme oxygenase-1 (HO-1) is involved in angiogenesis and tumor metastasis. Matrix metalloproteinases (MMPs) are key enzymes in the degradation of extracellular matrix, and their expression may be dysregulated in lung cancer metastasis. In this study, we investigated the anti-invasive mechanism of resveratrol in lung cancer cells. HO-1 was shown to be elevated (~4.7-fold) in lung cancer tumor samples as compared with matched normal tissues. After treatment of lung adenocarcinoma cell line A549 cells with resveratrol (50 μM) for 24 h, the migratory and invasive abilities (38 and 30% inhibition, respectively) of A549 cells were significantly reduced. Resveratrol significantly inhibited HO-1-mediated MMP-9 (35% inhibition) and MMP-2 (28% inhibition) expression in lung cancer cells. Nuclear factor (NF)-κB inhibitor induced a marked reduction in MMP-9 and MMP-2 expression, suggesting NF-κB pathway could play an important role. Furthermore, HO-1 inhibition and silencing significantly suppressed MMPs and invasion of lung cancer cells. Our results suggest that resveratrol inhibited HO-1 and subsequently MMP-9 and MMP-2 expression in lung cancer cells. The inhibitory effects of resveratrol on MMP expression and invasion of lung cancer cells are, in part, associated with the HO-1-mediated NF-κB pathway.

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Abbreviations: CO, carbon monoxide; **CoPPIX,** cobalt protoporphyrin IX; **ECM,** extracellular matrix; **EMSA,** electrophoretic mobility shift assay; **FBS,** fetal bovine serum; **HO-1,** heme oxygenase-1; **MMP,** matrix metalloproteinase; **NF,** nuclear factor; **NSCLC,** non-small cell lung cancer; **PDTC,** pyrrolidine dithiocarbamate; **siRNA,** small interfering RNA; **SnPPIX,** tin protoporphyrin IX

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1 Introduction

Resveratrol, 3,5,4′-trihydroxystilbene, is a natural polyphenol present in various plants that have been studied extensively in cardioprotection, inhibition of platelet aggregation, anti-inflammatory properties, antioxidant capabilities, and vasorelaxant activities [1]. Resveratrol can suppress proliferation, invasion, and apoptosis of various cancer cells by regulating nuclear factor (NF)-κB activities [2]. In addition, it also exhibits anticancer properties in different tumor cell types, including breast, prostate, stomach, colon, pancreas, and thyroid cancers [1, 3–5]. The highly potent inhibitory effects of resveratrol against tumorgenesis might suggest that it is an efficient agent for chemoprevention [6].

Lung cancer is the leading cause of cancer mortality. The progression of cancer from benign and delimited growth to invasive and metastatic growth is the major cause of poor clinical outcome in lung cancer patients [7]. Non-small cell lung cancer (NSCLC) account for 80–85% of all lung cancers [8]. Recent improvements in our understanding of the molecular pathologies of NSCLC have enabled the development of new, rationally designed, targeted therapeutics such as inhibition of tumor cell proliferation and angiogenesis; induction of cell apoptosis; and enhancement of the antitumor immune response by interaction with receptors, ligands, signaling molecules, or gene products that are pivotal in tumor growth, development, and metastasis [9].

Invasion and metastasis are fundamental properties of malignant cancer cells. The degradation of extracellular matrix (ECM), which exerts biochemical and mechanical barriers to cell movement, has been shown to be an important biological process in the metastasis of cancer cells [10]. ECM degradation and remodeling require the action of extracellular proteinases, among which matrix metalloproteinases (MMPs) have been shown to play an essential role. Recent reports have suggested that the type IV collagenases or gelatinases (MMP-2 and MMP-9) are critical for cell migration leading to invasion and metastasis of cancer [11, 12]. The synthesis and secretion of MMPs can be stimulated by a variety of stimuli, including cytokines, during various pathological processes such as tumor invasion, atherosclerosis, inflammation, and rheumatoid arthritis [13, 14]. On the basis of reports from several different groups, it has been concluded that expression of MMPs can be induced by cytokines *via* activation of intracellular signaling pathways and transcription factors such as NF-κB [13, 15].

Heme oxygenase-1 (HO-1) catalyzes heme to carbon monoxide (CO) and bilirubin with a concurrent release of iron. There has been accumulating evidence supporting the role of HO-1 in the cytoprotection against oxidative stress and other stimuli [16]. However, several studies also demonstrated that HO-1 overexpression is implicated in the pathogenesis and progression of several types of malignancies [17, 18]. In tumor-bearing mice, overexpression of HO-1 caused increased viability, proliferation, and angiogenic potential of melanoma cells and augmented metastasis [19]. Moreover, pharmacological inhibitors of HO-1 have anticarcinogenic effects in several tumor models [20]. MMPs are involved not only in ECM remodeling and degradation but also in cell migration, angiogenesis, and wound healing [21]. Among them, MMPs are often upregulated in cancer and are hence known as a predictive marker of mammary cancer progression [22]. However, precise mechanisms underlying modulation of HO-1 and MMPs in human lung cancer remain incompletely clarified.

HO-1 and MMPs are considered to play important roles in tumor metastasis. However, little information is available on the effect of HO-1 on the regulation of MMP expression and related molecular mechanisms. In addition, literature regarding the anti-invasive effects of resveratrol or its analogs (3,5,2′,4′-tetramethoxy-*trans*-stilbene [23], 3,4,5,4′-tetrahydroxystilbene [24], 3,5,4′-trimethoxy-*trans*-stilbene [25], *etc.*) on lung cancer cells has been limited. In this study, we investigated whether resveratrol could affect HO-1 expression and suppress the expression MMPs during lung cancer cell invasion.

2 Materials and methods

2.1 Culture of NSCLC A549 cells

Lung adenocarcinoma cell line A549 cells (ATCC number CCL-185) were cultured in flasks in F12K growth medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL of penicillin and 100 pg/mL of streptomycin. The cells were cultured at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Cell viability was determined with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The chemical drugs used in this study were purchased form Sigma Chemical (St Louis, MO, USA). The reduction in optical density caused by drugs was used as a measurement of cell viability, normalized to cells incubated in control medium, which were considered 100% viable. The drugs (*trans*-resveratrol (3,4′,5-Trihydroxy-*trans*-stilbne), pyrrolidine dithiocarbamate (PDTC), cobalt protoporphyrin IX (CoPPIX), tin protoporphyrin IX (SnPPIX), *etc.*) used did not significantly influence cell viability (>90%).

2.2 *In vitro* invasion and migration analyses

Cellular invasion was quantified using a modified Matrigel Boyden chamber assay. The BD BioCoat Matrigel invasion chamber (BD Biosciences, Bedford, MA) was used according to the manufacturer's instructions. A549 cells (4×10^4) in serum-free media were seeded onto Matrigel-coated filters. In the lower chambers, 5% FBS was added as a chemoattractant. After incubation for 24 h, the membrane was washed briefly with PBS and fixed with 4% *para*-formaldeyde. The upper side of membrane was wiped gently with a cotton ball. The membrane was then stained using hematoxylin and removed. The magnitude of A549 cells migration was evaluated by counting the migrated cells in six random clones under high-power (×100) microscope fields.

The migration ability of A549 cells were assayed in a monolayer denudation assay [26]. The confluent cells were wounded by scraping with a 100-μL pipette tip, which denuded a strip of the monolayer that is 300 μm in diameter. The cultures were washed twice with PBS, then the medium supplemented with 5% FBS was added and the rate of wound closure was observed after 24 h. The cells that migrated into the denuded area were photographed and the areas were analyzed.

2.3 Western blot analysis

Cells were lysed with lysis buffer (0.5 M NaCl, 50 mM Tris, 1 mM EDTA, 0.05% SDS, 0.5% Triton X-100, 1 mM PMSF, pH 7.4, for 30 min at 4°C, then the cell lysates were centrifuged at 4000 *g* for 30 min at 4°C. Protein concentrations in the supernatants were measured using a Bio-Rad protein determination kit (Bio-Rad, Hercules, CA). The supernatants were subjected to 10% SDS-PAGE, then transferred for 1 h at room temperature to polyvinylidene difluoride membranes (NEN), which were then treated for 1 h at room temperature with PBS containing 0.05% Tween 20 and 2% skimmed milk and incubated separately for 1 h at room temperature with mouse anti-human-HO-1 (Santa Cruz), or goat anti-human MMP2 and MMP9 antibodies (Santa Cruz). After washing, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated rabbit anti-goat or mouse IgG. Immunodetection was performed using chemiluminescence reagent plus (NEN) and exposure to Biomax MR Film (Kodak).

2.4 Nuclear extract preparation and electrophoretic mobility shift assay

Nuclear protein extracts were prepared before electrophoretic mobility shift assay (EMSA). Briefly, after washing with PBS, the cells were scraped off the plates in 0.6 mL of ice-cold buffer A 10 mM HEPES, pH 7.9, 10 mM KCl, 1 mM DTT, 1 mM PMSF, 1.5 mM $MgCl_2$, and 2 μg/mL each of aprotinin, pepstatin, and leupeptin. After centrifugation at 300 g for 10 min at 4°C, the cells were resuspended in buffer B (80 μL of 0.1% Triton X-100 in buffer A), left on ice for 10 min, then centrifuged at 12 000 g for 10 min at 4°C. The nuclear pellets were resuspended in 70 μL of ice-cold buffer C (20 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 0.42 M NaCl, 1 mM DTT, 0.2 mM EDTA, 1 mM PMSF, 25% glycerol, and 2 μg/mL each of aprotinin, pepstatin, and leupeptin), then incubated for 30 min at 4°C, followed by centrifugation at 15 000 g for 30 min at 4°C. The resulting supernatant was stored at −70°C as the nuclear extract. Protein concentrations were determined by the Bio-Rad method. The NF-κB probe used in the gel shift assay was a 31-mer synthetic double-stranded oligonucleotide (5′-ACA AGG GAC TTT CCG CTG GGG ACT TTC CAG G-3′; 3′-TGT TCC CTG AAA GGC GAC CCC TGA AAG GTC C-5′) containing a direct repeat of the κB site. For the EMSA, the digoxigenin (Dig) gel shift kit for 3′-end labeling of oligonucleotides (Roche, Indianapolis, IN) was used for nuclear protein-DNA binding assays. To confirm that the presence of bands is specific to NF-κB, unlabeled oligonucleotide controls and specific p65 antibody were added to the binding mixture for supershift assay.

2.5 Tumor sample collection

NSCLC and corresponding normal tissues were collected from non-selected patients who underwent surgical resection at the Division of Thoracic Surgery, Department of Surgery, Kaohsiung Medical University Hospital from 2004 to 2007. Lung tumors were classified by histological type, grade, and stage according to WHO standards [27]. Informed consent was obtained from the patients to participate in the study. The study was approved by the Ethical Review Board for Research (KMUH-IRB-940292 and KMUH-IRB-950276) at Kaohsiung Medical University Hospital, Taiwan.

2.6 HO-1 silencing in A549 Cells

The small interfering RNA (siRNA), a specific double-stranded 21-nucleotide RNA sequence homologous to the target gene, was used to silence HO-1 expression. siRNA for HO-1 and Negative Control ♯1 siRNA were designed and synthesized using the computer software from Ambion (Austin, TX) and Silencer™ siRNA construction kit from Ambion according to the manufacturer's instructions. Inhibition of HO-1 protein expression was assessed by immunoblot analysis following transfection of A549 with HO-1-siRNA. Briefly, A549 cells were grown in 100-mm dishes and transiently transfected with 20 nM siRNA using 8 μL of siPORT Amine (Ambion) in a total transfection volume of 0.5 mL of medium. After incubation at 37°C, 5% CO_2 for 5 h, 1.5 mL of normal growth medium was added and incubated with A549 cells for 48 h.

2.7 Statistical analyses

Results are expressed as the mean ± SEM. Data were analyzed by ANOVA and subsequently by Dunnetts' test. All statistics were calculated using SigmaStat version 3.5 (Systat Software, USA) and a p value of less than 0.05 was considered statistically significant.

3 Results

3.1 Effect of resveratrol on the viability of A549 cells

The cytotoxicity of resveratrol on A549 cells was initially determined with the MTT assay. The decrease in absorbance in this assay could either be a reduction in cell proliferation or consequence of cell death. The A549 cells were treated with resveratrol at various concentrations (12.5, 25, 50, and 100 μM) for 6–24 h. The results showed that treatment with resveratrol at different concentrations ranging from 12.5–100 μM exhibited no cytotoxic effects on the A549 cells for 24 h. Therefore, these concentrations of resveratrol and treatment time without cytotoxicity on A549 cells were used for subsequent experiments.

3.2 Resveratrol inhibits migration and invasion of A549 cells

To explore the potential anti-invasive effects of resveratrol, the migration and invasion assays were performed on A549. Untreated group (0 μM resveratrol) served as control (100%). Treatment of cells with resveratrol at various concentrations (12.5–100 μM) for 24 h reduced their migratory and invasive abilities in a dose-dependent manner. After treatment with resveratrol (50 and 100 μM) for 24 h, the migratory (38 and 56% inhibition, respectively) and invasive (30 and 42% inhibition, respectively) abilities of A549 cells were significantly ($p < 0.05$) reduced, as compared with that of the control (Figs. 1A and B). These results suggested that resveratrol might be an effective inhibitor for the invasion of A549 cells.

3.3 Resveratrol inhibits MMP-2, MMP-9, and HO-1 expression

The invasion of tumors is a highly complex and multistep process that requires a cancer cell to modulate its ability to degrade ECM, migrate, proliferate, and stimulate angiogenesis [28]. MMPs have been recognized as major critical molecules assisting tumor cells during invasion [12, 29]; because of stimulatory role of HO-1 in angiogenesis, HO-1 can also facilitate tumor metastasis [28]. Thus, the effects of resveratrol on the expression of MMPs and HO-1 on A549 cells were analyzed to clarify if changes in their expression were involved in resveratrol-inhibited invasion of A549 cells. The cells were treated with various concentrations of resveratrol (12.5–100 μM) for 24 h and the total proteins were analyzed using Western blot analysis. Untreated group served as control for the experiments in which MMP-2, MMP-9, and HO-1 expression was determined. It was found that MMP-2, MMP-9, and HO-1 were decreased in a dose-dependent manner by resveratrol treatment (Fig. 2A).

3.4 Resveratrol inhibits MMP-2 and MMP-9 through suppression NF-κB activity

NF-κB-mediated expression of MMPs and NSCLC metastasis has been demonstrated in previous studies [30]. Therefore, we examined the effects of the well-known NF-κB inhibitor PDTC on the expressions of MMPs and HO-1. A549 cells were treated with PDTC (25–150 μM) for 24 h,

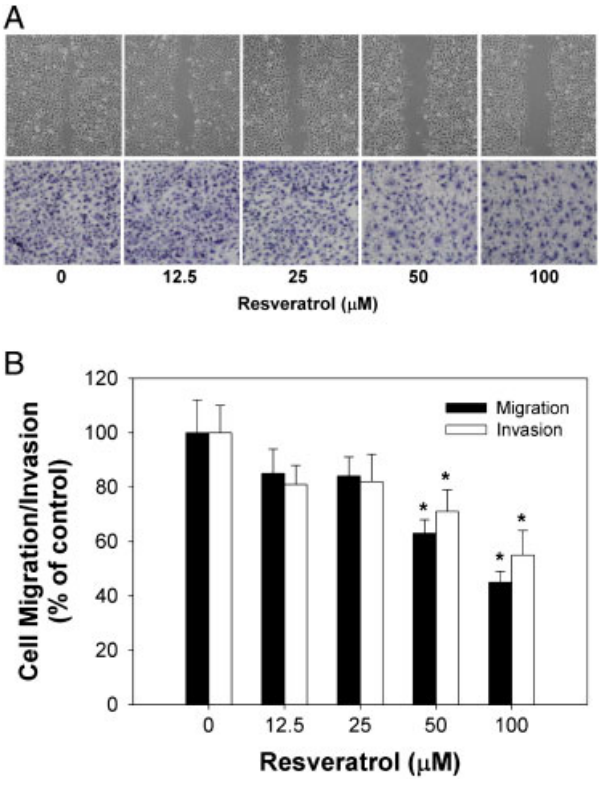

Figure 1. Effect of resveratrol on tumor cell invasiveness and migration. (A) Cellular invasiveness and migration were determined by wound scratch assays and Matrigel-coated Boyden chamber assays, respectively. A549 cells were incubated in medium containing various concentrations (12.5, 25, 50, or 100 μM) of resveratrol for 24 h. (B) The cells that migrated into the denuded area were photographed and the areas were analyzed; the magnitude of A549 cells invasion was evaluated by counting the migrated cells in six random clones under high-power (×100) microscope fields. Data (mean ± SEM of three independent experiments) are expressed as the percentage of control.

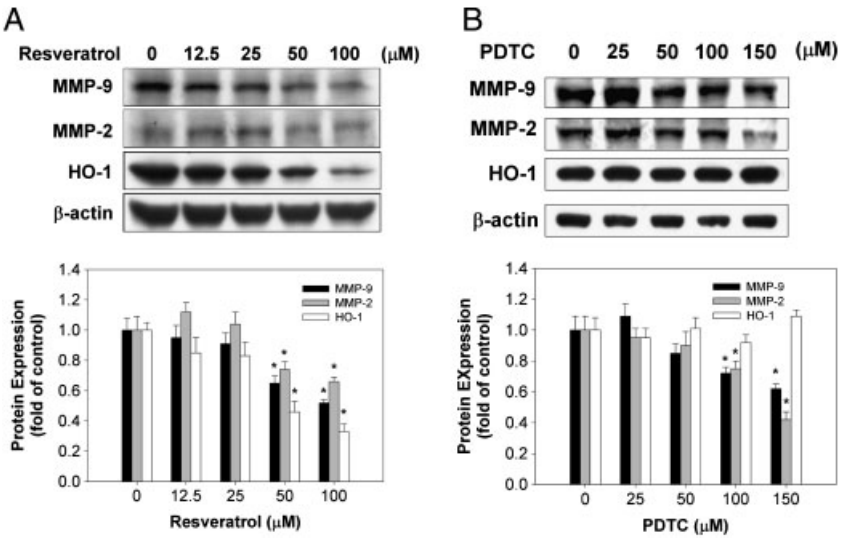

Figure 2. Effect of resveratrol and PDTC on MMP-9/MMP-2 and HO-1 expression. (A) Resveratrol dose-dependently inhibited MMP-9, MMP-2 and HO-1 protein level. (B) NF-κB inhibitor PDTC dose-dependently inhibited MMP-9 and MMP-2 but not HO-1 expression. Densitometric analysis was conducted with a software to semiquantify Western blot data. Three independent experiments gave similar results. Data are means ± SEM from three independent experiments. $^{*}p < 0.05$, compared with untreated control group.

MMP-9/MMP-2 and HO-1 expressions were evaluated by Western blot analysis. As shown in Fig. 2B, PDTC decreased MMP-2 and MMP-9 expressions in a dose-dependent manner. However, PDTC has no specific inhibition of HO-1 expression.

We next examined the effects of resveratrol on the activation of NF-κB in A549 cells. The cells were treated with resveratrol (12.5–100 μM) for 24 h, and the nuclear extracts were analyzed by Western blot analysis and gel-shift assay. The results showed that NF-κB was consistently activated in A549 cells; resveratrol treatment decreased both the protein levels of p65 and p50 in nucleus as well as DNA-binding activity of consensus NF-κB (Fig. 3A) in a dose-dependent manner. To confirm that the presence of bands is specific to NF-κB, unlabeled oligonucleotide controls and specific p65 antibody were added to the binding mixture for supershift assay (Fig. 3B). These results imply that resveratrol is unique in its ability to inhibit NF-κB activation and suppress expressions of MMPs.

3.5 Involvement of HO-1 in NF-κB-mediated MMP-2 and MMP-9 expression

According to the recent study [31] and our clinical pilot analysis (Fig. 4A), HO-1 was shown to be evaluated in lung cancer tumor samples as compared with matched normal tissues from patients. We next examined the possible role of HO-1 in NF-κB-mediated MMP-2/MMP-9 expression and subsequent cell invasion. The HO-1 inducer CoPPIX slightly induced activation of NF-κB whereas SnPPIX, a competitive inhibitor of HO-1 activity, potently inhibited NF-κB activation (Fig. 4B). In the meantime, CoPPIX significantly induced A549 cell migration and invasion, whereas SnPPIX inhibited cell metastasis (Fig. 4C). Additionally, we used a siRNA-expressing plasmid to induce HO-1 gene silencing. A549 cells were transfected with an HO-1-targeting siRNA expression vector. The siRNA approach resulted in high silencing efficacies for HO-1. We achieved more than 50% protein silencing, which was confirmed by Western blot analysis (Fig. 5A). Silencing of HO-1 significantly attenuated MMP-2/MMP-9 (Fig. 5B) as well as cellular invasiveness and migration of A549 cells (Fig. 5C). For these experiments, cells that were transfected with a vector encoding a non-targeting siRNA served as controls. Modulation of HO-1 expression had no effect on cell viability, which was confirmed by cellular proliferation assays (data not shown). These data suggest that resveratrol-inhibited expression of MMPs and invasion of NSCLC cells are, in part, associated with the down-regulation of HO-1-mediated NF-κB signaling pathway.

4 Discussion

In the study, we showed for the first time that resveratrol inhibits MMP-9/MMP-2 expression and subsequently human lung adenocarcinoma cell metastasis in a HO-1-related mechanism. Moreover, HO-1 inhibitor or silencing induced the downregulation of MMP-9/MMP2, which is in part due to the suppression of NF-κB-dependent signaling pathway. These findings show that resveratrol might act as a therapeutic agent in the inhibition of cancer progression and provide a novel mechanistic insight into the potential effects of resveratrol on the suppression of tumor invasion and metastasis.

Tumor angiogenesis, invasion, and metastasis require controlled degradation of ECM, and increased expression of MMPs is associated with tumor invasion and metastasis of malignant tumors [32]. HO-1 modulation has been shown to correlate with MMP expression and tumor invasion [18, 33]. Although resveratrol has been indicated as a strong anti-carcinogenic compound, its effect on HO-1-mediated MMP expression and cellular invasion is still unclear. We initially

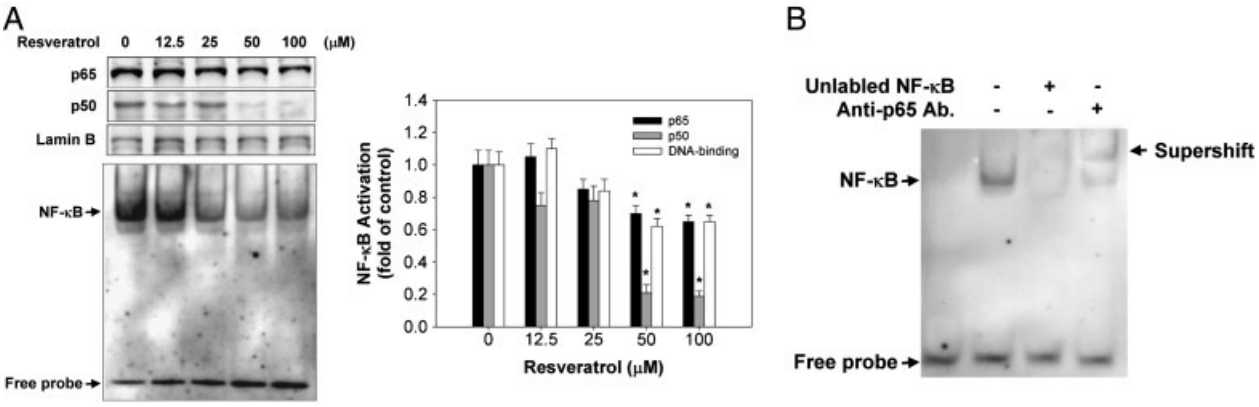

Figure 3. Effect of resveratrol on NF-κB activation. (A) Resveratrol dose-dependently inhibited nuclear translocation of p65/p50 and NF-κB activation. Lamin B was used as a loading control for nuclear protein. Densitometric analysis was conducted with a software to semiquantify Western blot and EMSA data. Three independent experiments gave similar results. Data are means ± SEM from three independent experiments. $^{*}p < 0.05$, compared with untreated control group. (B) To confirm that the presence of bands is specific to NF-κB, unlabeled oligonucleotide controls and anti-p65 antibody were also used for supershift in the separated studies.

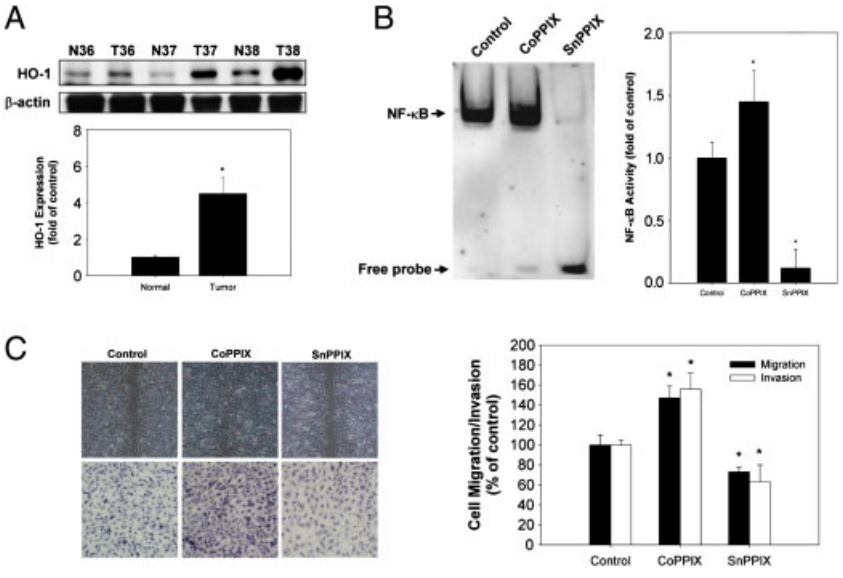

Figure 4. Expression of HO-1 in clinical lung cancer samples and the effect of HO-1 modulation in A549 cells. (A) HO-1 was shown to be elevated in lung cancer tumor samples as compared with matched non-tumor tissues from patients. (B) The HO-1 inducer CoPPIX slightly induced activation of NF-κB whereas HO-1 inhibitor SnPPIX potently inhibited NF-κB activation. (C) CoPPIX significantly induced A549 cell migration and invasion, whereas SnPPIX inhibited cell metastasis. Densitometric analysis was conducted with a software to semiquantify Western blot and EMSA data. Three independent experiments gave similar results. Data are means ± SEM from three independent experiments. $^{*}p < 0.05$, compared with untreated control group.

tested the possibility that resveratrol could inhibit the HO-1-mediated mechanism. Since HO-1-mediated mechanism could play an important role in the regulation of MMP expression [18, 33], we further tested the downstream NF-κB pathway. These results suggest that expression of MMPs is sensitive to the activation status of NF-κB. The HO-1 inducer CoPPIX induced activation of NF-κB whereas HO-1 inhibitor SnPPIX potently inhibited NF-κB activation; silencing of HO-1 and SnPPIX significantly attenuated MMPs. This suggests that the extent to which resveratrol inhibited HO-1-related NF-κB could be compromised. It is consistent with previous findings that resveratrol acts as a chemotherapeutic agent in targeting intracellular signaling molecules. Our findings provide a novel mechanistic insight into the potential effects of resveratrol on the suppression of tumor invasion and metastasis.

Investigations of the role of HO-1 seem to be important not only for better understanding of tumor growth regulation but also for clinical practice. HO-1 cleaves the prooxidant heme molecule into CO, ferrous iron, and biliverdin that is subsequently converted to bilirubin [34]. Strong expression of HO-1 is often observed in tissues with a high rate of cell proliferation such as adenocarcinoma, hepatoma, sarcoma, glioblastoma, melanoma, and squamous carcinoma [35–38]. Contradictory results published so far indicate that the role of HO-1 in regulation of the cell cycle is strongly tissue-type-dependent. In some models, for example in astroglia, pulmonary or proximal tubular epithelium, vascular smooth muscles, or breast carcinoma, activation of

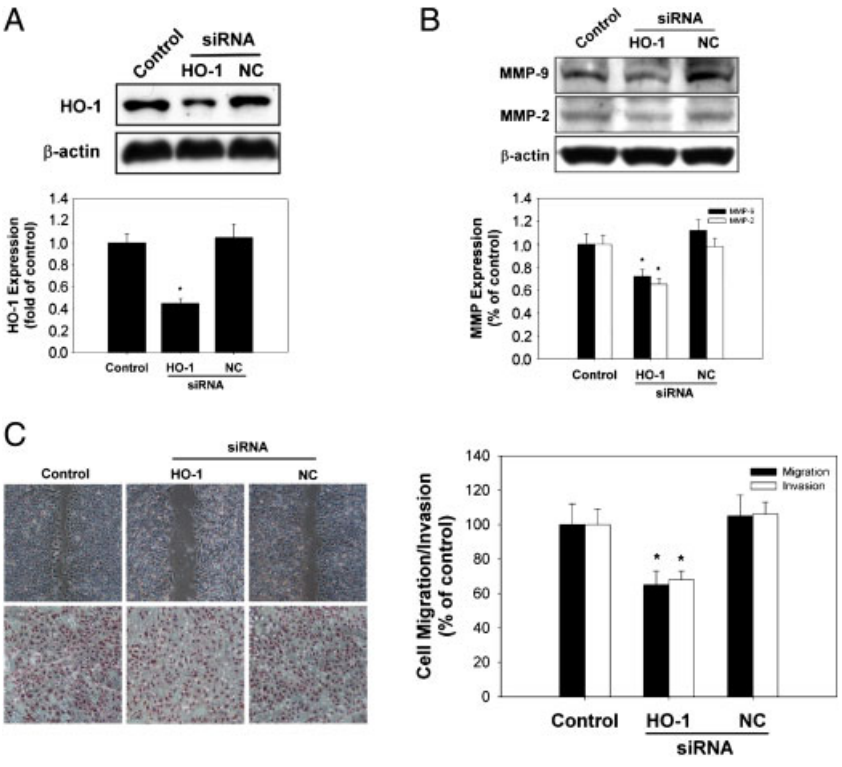

Figure 5. Effect of HO-1 silencing and overexpression on A549 cell invasiveness. (A) The siRNA approach resulted in high silencing efficacies for HO-1. (B) Silencing of HO-1 significantly attenuated MMP-2/MMP-9 and (C) cellular invasiveness and migration in A549 cells. Densitometric analysis was conducted with a software to semiquantify Western blot data. Three independent experiments gave similar results. Data are means ± SEM from three independent experiments. $^{*}p < 0.05$, compared with untreated control group.

HO-1 results in cell growth arrest [35–40]. In the other cell types, such as epidermal keratinocytes and vascular endothelium, pharmacological induction or genetic overexpression of HO-1 stimulates cell division [41–43]. Our study demonstrated that resveratrol inhibits MMP-9/MMP-2 expression and decreases invasiveness of human lung cancer cells through inhibition of HO-1 expression. These data agree with a previous study showing that 15-deoxy-$D^{12,14}$-prostaglandin J_2 upregulates the expression of HO-1 and subsequently MMP-1 in human breast cancer cells [18].

HO-1 has been shown to inhibit breast cancer invasion *via* suppressing the expression of MMP-9 [33]. More recently, resveratrol analog was found to inhibit invasion of human lung adenocarcinoma cells by decreasing MMP-2 expression [25]. There are still some discrepancies of the roles of HO-1 in tumor metastasis; further studies are required to confirm the real role of HO-1 in the metastasis of NSCLC, especially in different types of tumors. On the other hand, CO and biliverdin are released as by-products of HO-1-catalyzed heme degradation. The roles of the latter products of HO-1 activity in resveratrol-modulated MMP expression need to be encouraged to be investigated. Our *in vitro* invasion assay showed that inhibition of the endogenous HO-1 protein expression through the siRNA knockdown technique suppressed the cell invasion substantially. These findings suggest an association between HO-1 and tumor cell motility, invasion, and metastasis. Thus, our studies have shown that HO-1, as a potentially protection protein for tumor cells, might play an important role in the progression of NSCLC. Our observations provide new insights into the understanding of the HO-1 protein in cancer.

Resveratrol toxicity has been reported at concentrations above 10 μM in A549 cells [44]. Our results showed that no significant cytotoxic effect of resveratrol was found at a concentration below 100 μM. The deviation between the above results may be related to differences in cell passages and other experimental conditions (medium formula, FBS source, and drug batch number) or the different cytotoxic analysis methods. Resveratrol exists as two geometric isomers: *cis*- (Z) and *trans*- (E) forms. *Trans*-resveratrol in powder form was found to be stable under ''accelerated stability'' conditions of 75% humidity and 40°C in the presence of air [45], its content also stayed stable in the skins of grapes and pomace taken after fermentation and stored for a long period [46]. However, resveratrol should be handled carefully, because *trans*-resveratrol can undergo isomerization to the *cis* form under ultraviolet irradiation. Since *cis*-resveratrol is not commercially available, exposure to sunlight/daylight, intense white light, and ultraviolet light have been reported could transform the *trans*-resveratrol into *cis* in the solution [47].

In addition, although the biological positive effects of resveratrol are largely admitted, little is known about the transport and the distribution of resveratrol through the body. Due to its low water solubility [48], resveratrol must be bound to proteins and/or conjugated to remain at a high concentration in serum. Resveratrol can be bound by protein and the

presence of FBS can facilitate this level of solubility. Moreover, the efficiency of a therapeutic substance is related to its capacity to bind protein transporters [49]. Aerosol delivery of therapeutic agents has the potential of localizing the drugs specifically to the lung tissue, with a comparable or better pharmacokinetics as compared with oral, intravenous, or intraperitoneal delivery [50]. Aerosol delivery of resveratrol has not been studied extensively; however, clinical preparation and delivery remain issues, though an aerosol version would have obvious benefits.

In summary, the present study shows that resveratrol inhibits expression of MMP-9/MMP-2 and human lung adenocarcinoma cell metastasis by suppressing HO-1. Moreover, HO-1 inhibitor or silencing induced the down-regulation of MMP-9/MMP2, which is in part due to the suppression of NF-κB-dependent signaling pathway. The findings in this study provide new insights into the understanding of the molecular mechanism involved in the progression and prognosis of NSCLC.

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The authors have declared no conflict of interest.

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