Research Article

Resveratrol inhibits migration and invasion of human breast-cancer cells

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Metastasis is the primary cause of death from breast cancer. Cell migration and invasion play important roles in neoplastic metastasis. The insulin-like growth factor (IGF-1) stimulates cell migration through activation of PI-3K/Akt signaling pathway. IGF-1 induces the tumorigenicity of many types of cancer cells and is critical for metastatic cell spread in estrogen receptor (ER)-negative breast-cancer cells. Matrix metalloproteinase-2 (MMP-2) is a key enzyme in the degradation of extracellular matrices and its expression has been dysregulated in breast cancer invasion and metastasis. Resveratrol exhibited potential anticarcinogenic activities in several studies. However, the inhibitory effects of resveratrol on the expression of MMP-2, migration and invasion of breast-cancer cell have not been demonstrated yet. In the present study, we investigated the anti-invasive mechanism of resveratrol in human breast cancer MDA-MB 435cells. Here, we showed that IGF-1 is a potent stimulant of the migration of ER-negative human breast-cancer cells. Resveratrol could inhibit IGF-1-mediated cell migration of MDA-MB 435 *in vitro.* The inhibitory effect of resveratrol was mediated in part through the suppression of the activation of PI-3K/Akt signaling pathway. Furthermore, IGF-1-mediated expression of MMP-2 was significantly inhibited by resveratrol in concomitance with alteration of cell invasion.

Keywords: Breast-cancer cells / Invasion / Matrix metalloproteinase / Migration / Resveratrol

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1 Introduction

Breast cancer is one of the leading causes of malignancy-related death in the world [1]. Despite successful treatment of the primary malignancy, relapse and subsequent metastatic spread can still occur at distant sites, including bone, lung, liver, and brain [2]. The insulin-like growth factor 1 receptor (IGF-1R) is a ubiquitous, transmembrane tyrosine kinase that has been implicated in different growth-related and growth-unrelated processes critical for the development and progression of malignant tumors, such as proliferation, survival, and anchorage-independent growth, as well as cell adhesion, migration, and invasion [3 – 5]. The IGF-1R is overexpressed (up to 14-fold) in estrogen receptor (ER)-positive breast-cancer cells compared to its levels in normal epithelial cells [3 – 6]. IGF-1R ligand, IGF-1, is strong mitogen for many hormone-dependent breast-cancer cell lines and has been found in the epithelial and/or stromal component of breast tumors [6]. Importantly, higher levels of circulating IGF-1 predict increased breast cancer risk in premenopausal women [5]. *In vitro*, activation of the IGF-1R, especially the IGF-1R/IRS-1/PI-3K pathway in ER-positive breast-cancer cells, counteracts apoptosis induced by different anticancer treatments or low concentrations of hormones [3, 6]. The possible mechanisms could be mediated through the up-regulation of surviving to maintain cell proliferation and cell viability in most human cancers [7]. Several studies also indicated that surviving could modulate the expression of tumor suppressor p53 inhibition effect on cell-cycle progression and prevent apoptosis [8, 9]. However, ER-negative tumors and cell lines, often exhibiting less differentiated, mesenchymal phenotypes, express low levels of the IGF-1R and often decreased levels of insulin receptor substrates (IRS-1) [3, 6]. Notably, these cells do not respond to IGF-1 with growth [3, 6]. Despite the lack of

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Abbreviations: **ER**, estrogen receptor; **IGF-1**, insulin-like growth factor; **IRS-1**, insulin receptor substrates; **MI**, migration index; **MMP-2**, matrix metalloproteinase-2

IGF-1 mitogenic response, the metastatic potential of ER-negative breast-cancer cells can be effectively inhibited by different compounds targeting the IGF-1R [10]. Previous study indicated that IGF-1 ligand activated IGF-1R signaling and up-regulated matrix metalloproteinase (MMP) activity [11]. MMP are required for the breakdown of the extracellular matrix and play important roles in the progresses of cancer invasion and metastasis. Malignant progression is frequently associated with up-regulated production and/or activity of one or several MMP, a family of extracellular matrix-degrading enzymes, which have been implicated in tumor invasion by *in vitro* and *in vivo* studies [12, 13]. The 72-kDa type IV collagenase, MMP-2, is thought to play a critical role in tumor cell invasion by facilitating the degradation of basement membrane type IV collagen [14]. Elevated levels of MMP-2 have been correlated with malignancies of several human cancers including breast, oral, gastric, bladder, and pancreatic cancers [15 – 19].

Blockade of the IGF-1R in MDA-MB-231 cells by an anti-IGF-1R antibody reduced migration *in vitro* and tumorigenesis *in vivo*, and expression of a soluble IGF-1R in MDA-MB-435 cells inhibited adhesion on the extracellular matrix and impaired metastasis in animals [6, 10]. These observations suggested that in ER-negative cells, some functions of the IGF-1R must be critical for metastatic cell spread.

Various polyphenolic compounds of dietary origin are known to inhibit carcinogenesis and exert anti-cancer activities responsible for health benefits [20, 21]. The polyphenol resveratrol, a phytoalexin present in grapes and red wine, is known to be associated with a decreased risk for cancer and other diseases [22 – 24]. However, the inhibitory effects of resveratrol on the migration and invasion of breast-cancer cells have not been demonstrated yet. Therefore, in the present study, we investigated the molecular mechanisms of resveratrol. Here, we demonstrated that IGF-1 induced the migration of ER-negative MDA-MB-435 breast-cancer cells via the activation of PI-3K signaling pathway. Resveratrol inhibited cellular migration in part through the inhibition of PI-3K/Akt signaling pathway. Furthermore, resveratrol suppressed invasion of breast-cancer cells *in vitro*.

2 Materials and methods

2.1 Reagents and antibodies

Resveratrol, fibronectin, DMSO, Wortmannin (specific inhibitor of PI-3K), PD098059 (specific inhibitor of MEK), gelatin, anti-β-actin antibody were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant IGF-1 protein and anti-phosphorylated Akt polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Anti-MMP-2 antibody was purchased from Cell Signal (Danvers, MA). L-15 medium and PBS were purchased from Invitrogen (Carlsbad, CA).

2.2 Cell culture

Human breast-cancer cell line MDA-MB 435 was purchased from American Type Culture Collection (Rockville, MD). Briefly, human breast-cancer cells were maintained at 37°C in 5% CO_2 and grown to confluency using 10% fetal bovine serum (FBS) L15-supplemented media.

2.3 Supplementation with resveratrol

Resveratrol was dissolved in DMSO at a concentration of 100 mM and stored at −20°C. Immediately before the experiment, the stock solution was added to the cell culture medium, as described previously. Human breast-cancer cells were incubated with different concentrations (0, 10 and 20 μM) of resveratrol for different periods. For efficient uptake of resveratrol by human breast-cancer cells, resveratrol was incorporated into FBS for 30 min and mixed with L-15 medium. The final concentration of DMSO was 0.01% v/v and 0.02% v/v for final concentration 10 and 20 μM, respectively. After incubation periods, cells were washed with PBS) prior to induction of cell migration or invasion by IGF-1.

2.4 Cell-motility colloidal gold phagokinetic assays

Cell migration on fibronectin was examined by using the migration track assay, as previously described for computer-assisted analysis. Briefly, approximately 1500 cells were plated onto coverslips coated with 10 μg fibronectin and treated with 10 ng/mL IGF-1 in the presence of 0, 10, and 20 μM resveratrol. Human breast-cancer cells were allowed to migrate for 12 h. At the end of the assay, cells were fixed and migration was examined under dark field optics and photographed. Twenty randomly selected and non-overlapping fields under each experimental condition were analyzed with an attached camera and a computer using the NIH Image 1.6 analyzer program (Scion, Frederick, MD). The system calculated the percentage of the total field area viewed by the camera that was consumed with linear cell migration tracks.

2.5 Western blot analysis

Human breast-cancer cells were stimulated with IGF-1 (10 ng/mL) in the presence of 0, 10, and 20 μM resveratrol for 30 min. Cells were lysed in a buffer containing : 1X PBS, 1% Ipegal CA-630 (Sigma), 0.5% sodium deoxycholate, 0.1% SDS with 100 μM of PMSF, aprotinin and specific phosphatase inhibitors, sodium orthovanadate. Cell lysates were cleared by centrifugation. Total cell lysates

were fractioned on 10% SDS-PAGE, transferred to nitrocellulose membrane, and blotted with anti-phosphorylated Akt antibodies (0.1 μg/mL) according to the manufacturer's instructions. The blots were stripped and reprobed with anti-β-actin antibody as loading control. MMP-2 was measured by using the same procedure described above.

2.6 Gelatin zymography

Gelatin zymography was performed for both control and IGF-1 treatment groups as follows [25]. Gels (SDS–PAGE, 7.5%) were co-polymerized with 0.1% gelatin. For each sample, equal amount of protein (100 μg) was loaded. Electrophoresis was carried out using the minigel slab apparatus Mini Protean 2 (Bio-Rad) at a constant voltage of 150 V, until the dye reached the bottom of the gel. Following electrophoresis, gels were washed in renaturation buffer (2.5% Triton X-100 in 50 mM Tris–HCl pH 7.5) for 1 h in an orbital shaker. Then, the zymograms were incubated for 24 h at 37°C in incubation buffer (0.15 M NaCl, 10 mM $CaCl_2$, 0.02% NaN_3 in 50 mM Tris–HCl pH 7.5). Gels were then stained with CBB and destained with 7% methanol and 5% acetic acid. Areas of enzymatic activity appeared as clear bands over the dark background.

2.7 Cell-invasion assay

The invasion of tumor cells was analyzed in transwell Boyden chambers with a polyvinyl/pyrrolidone–free polycarbonate filter of 8-μm pore size [26]. Each filter was coated with 100 μL of a 1:10 diluted matrigel in cold L-15 medium to form a thin continuous film on the top of the filter. Human breast-cancer cells stimulated with IGF-1 (10 ng/mL) were added to each of triplicate wells in L-15 medium (50 000 cells/well). After incubation for 16 h, cells in ten randomly selected fields were counted. The number of cells invading the lower side of the filter was measured as invasion activity (invasion index).

2.8 Statistical analysis

We used quantitative methodology to determine the difference in the migration capability between experimental sets of migrating breast-cancer cells and control sets of breast-cancer cells. Similar analysis was used in the measurement of invasion index. In brief, statistical analyses of the differences in migration capability among triplicate sets of experimental conditions were performed using SPSS. Confirmation of difference in migration as being statistically significant requires rejection of the null hypothesis of no difference between mean migration indices obtained from replicate sets at the $p = 0.05$ level with the one-way ANOVA analysis.

3 Results

3.1 Resveratrol inhibited IGF-1-mediated cell migration of breast-cancer cells

In the current study, we investigated the role of resveratrol in the prevention of migration and invasion in estrogen-independent human breast-cancer cells. We first examined the inhibitory effects of resveratrol on the migration of human breast-cancer cells. Here, we showed that IGF-1 (10 ng/mL) significantly induced human breast-cancer cell migration. As shown in Fig. 1, on a fibronectin-coated colloidal gold substratum, the unstimulated cells made few migration tracks (Fig. 1A). However, the cells made markedly linear migration tracks, leaving behind black gold particle-free tracks under the stimulation of IGF-1 (10 ng/mL) (Fig. 1B) (location of the cell inside each track is indicated by an arrow). At a concentration of 10 μM, resveratrol almost completely suppressed IGF-1-induced migration in human breast-cancer cells (Fig. 1C). We also found that resveratrol blocked cell migration on fibronectin without compromising cell viability (data not shown). In the absence of IGF-1, 10 μM resveratrol further suppressed migration in human breast-cancer cell in comparison to the control group (Fig. 1D *vs*. 1A). To measure the level of cellular migration, MI were analyzed as described in Section 2. As shown in Fig. 1G, in the absence of resveratrol and IGF-1, MDA-MB 435 cell migrated and produced MI over 2. IGF-1 (10 ng/mL) significantly induced cell migration with MI over 9. However, in the presence of resveratrol (10 μM), cell migration was suppressed and the MI were reduced dramatically in human breast-cancer cells. These results indicated that resveratrol could effectively inhibit breast-cancer cell migration. It also suggested that resveratrol -sensitive cellular targets played an important role in the IGF-1-driven cell migration in human breast cancer MDA-MB 435 cells.

To investigate which important signaling molecules involved in the IGF-1-mediated cell migration, different specific inhibitors were used to examine the molecular mechanisms of action. Since PI-3K/Akt and MAPK/ERK 1/2 signaling pathways are known to be involved in cell motility [27, 28], we first tested the role of ERK 1/2 in MDA-MB 435 cell migration under the IGF-1 stimulation. To determine whether IGF-1-mediated cell migration of human breast-cancer cells could be regulated by the downstream ERK 1/2 signaling pathway, the MDA-MB 435 cell migration was assessed by using MEK1-specific inhibitor PD098059. As shown in Figs. 1E and G, treatment of human breast-cancer cells with 10 μM PD098059 could not inhibit IGF 1-mediated migration of human breast cancer. This finding suggests that ERK 1/2 pathway is not involved in the IGF-1-mediated migration of human breast-cancer cells.

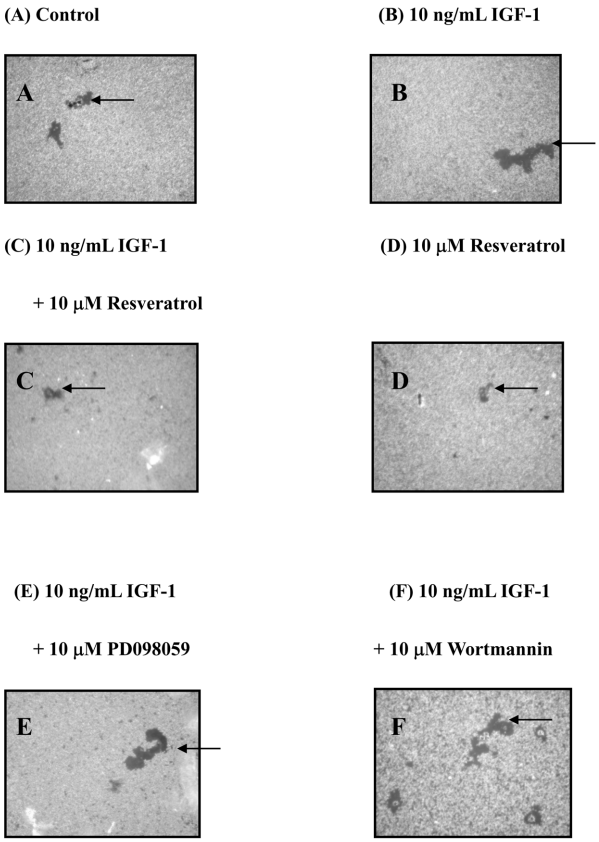
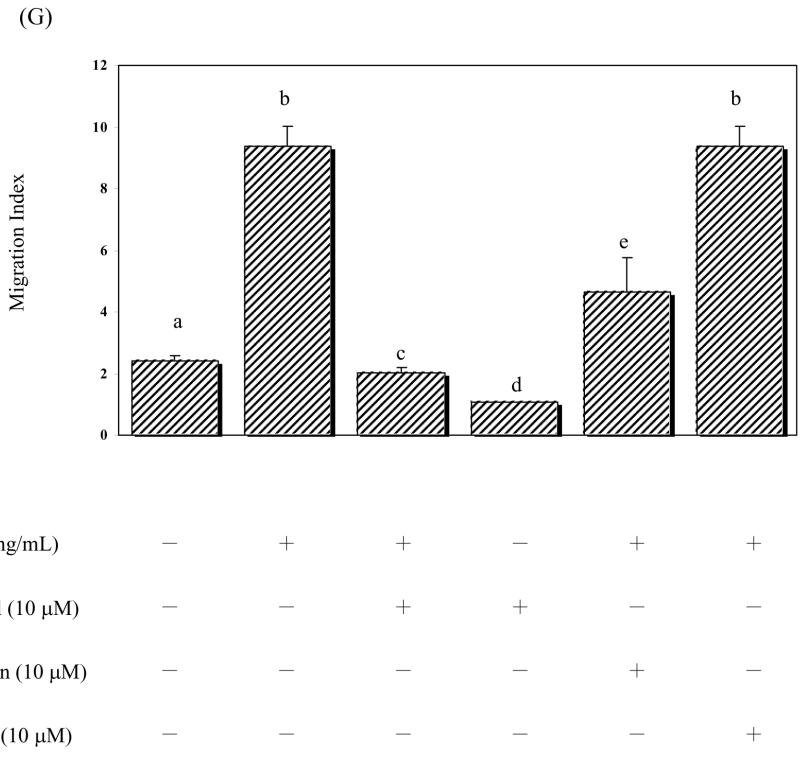


Figure 1. Resveratrol inhibited IGF-1-mediated cell migration of breast-cancer cells. Human breast-cancer cell MDA-MB 435, cultured in L-15 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation, and resuspended in L-15 medium. Approximately 1500 cells were seeded on each prepared fibronectin-coated gold salt-covered coverslips. The human breast-cancer cell were then stimulated with IGF-1 (10 ng/mL) in L-15 medium without or with 10 μM resveratrol for 12 h until measurement of cell migration. The incubation was stopped after 12 h by fixing the cells with 0.1% formaldehyde in PBS. The MDA-MB 435 were then stimulated with IGF-1 (10 ng/mL) in L-15 medium without or with 10 μM specific PI-3K inhibitor (Wortmannin), 10 μM specific MEK inhibitor (PD098059) for 12 h until measurement of cell migration. The incubation was stopped after 12 h by fixing the cells with 0.1% formaldehyde in PBS. The analysis of cell migration was described in Section 2. (A – F) Representative photographic images. (G) Migration index of human breast-cancer cells under the stimulation of IGF-1 with or without the presence of resveratrol, wortmannin, and PD098059. The migration indices (MI) were measured as described in Section 2. Data analysis from three separate experiments is shown as the mean ± SEM of migration index in 20 randomly selected fields in each culture dish. Different letters represent statistically significant difference, $p < 0.05$.



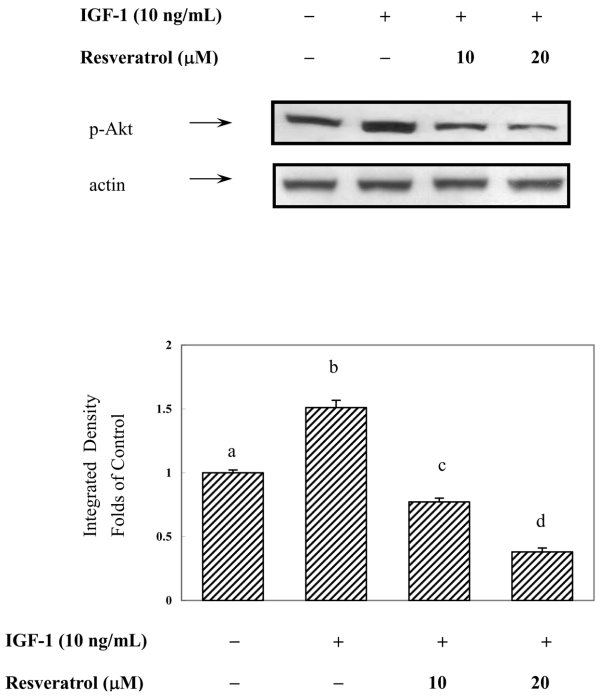


Figure 2. The inhibitory effects of resveratrol on IGF-1-mediated activation of PI-3K/Akt pathways in human breast-cancer cells. Confluent MDA-MB 435 cancer cells were incubated with 10 and 20 μM of resveratrol at 37°C for 2 h. After washing out the media, human breast-cancer cells were then stimulated by IGF-1 (10 ng/mL) for 30 min. Total cell lysates were blotted with anti-phosphorylated Akt primary antibody as described in Section 2. The values represent the amount of Akt-phosphorylation in human breast-cancer cells. The blots were stripped and reprobed with β-actin antibody as a loading control. The photographs of immunoreactive bands are indicated by arrows. The densitometric analysis is shown in the panel. Data analysis from three separate experiments is shown as the mean ± SEM of three independent experiments. Different letters represent statistically significant difference, $p < 0.05$.

We further tested the role of PI-3K in human breast-cancer cell migration under conditions of IGF-1 stimulation. To determine whether IGF-1-mediated migration of human breast-cancer cells could be regulated by the downstream PI-3K/Akt signaling pathway, migration of human breast-cancer cell was assessed by using specific PI-3K inhibitor, Wortmannin. As shown in Fig. 1F, treatment of human breast-cancer cells with 10 μM Wortmannin effectively inhibited IGF-1-mediated human breast-cancer cell migration. These results showed that blockade of PI-3K signaling significantly suppressed the human breast-cancer cell migration. Thus, PI-3K/Akt signaling pathway could be resveratrol-sensitive cellular targets during regulation of the IGF-1-driven cell migration in human breast cancer MDA-MB 435 cells. It is plausible that some other major pathways could be involved in the IGF-1-mediated migration of human breast-cancer cells.

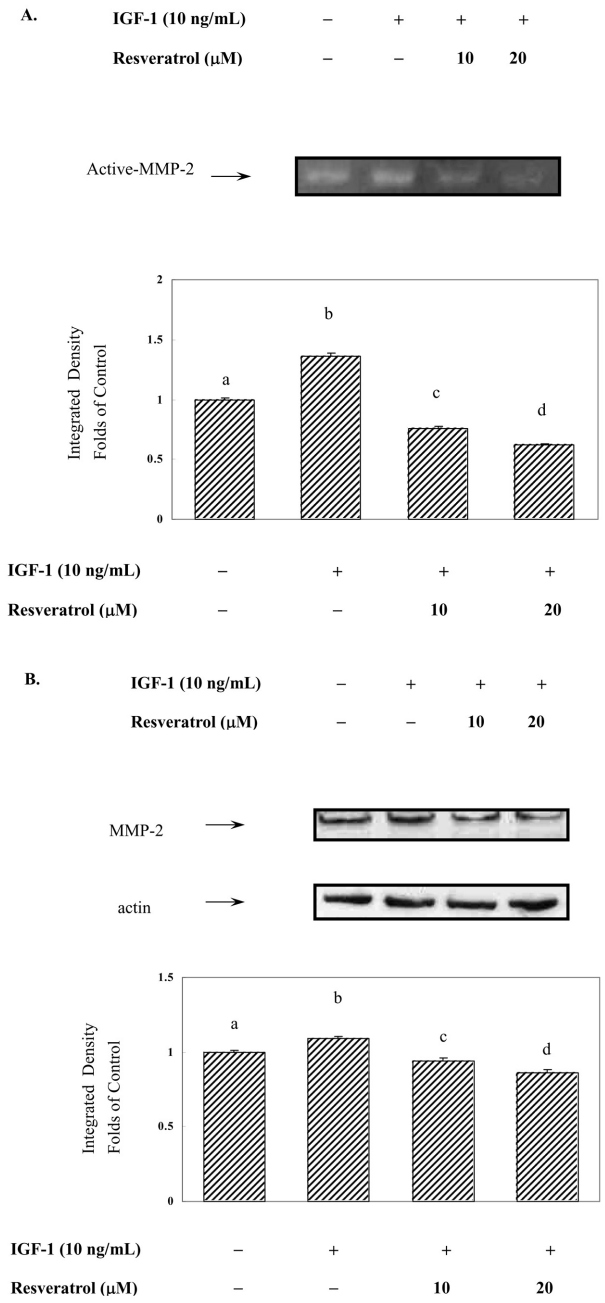


Figure 3. Resveratrol inhibited IGF-1-mediated expression of MMP-2 in human breast-cancer cells. Confluent MDA-MB 435 cancer cells were incubated with 10 and 20 μM of resveratrol at 37°C for 24 h in the presence of IGF-1 (10 ng/mL). After removing the supernatant of breast-cancer cells, total cell lysates of MDA-MB 435 cells were prepared according to description in Section 2. Supernatants of breast-cancer cells were loaded into gelatin-containing 10% gel. After incubation and staining of gelatin gel, the photographs of zymogram bands are noted with arrows. The densitometric analysis is shown. Data analysis from three separate experiments is shown as the mean ± SEM of three independent experiments. Different letters represent statistically significant difference, $p < 0.05$.

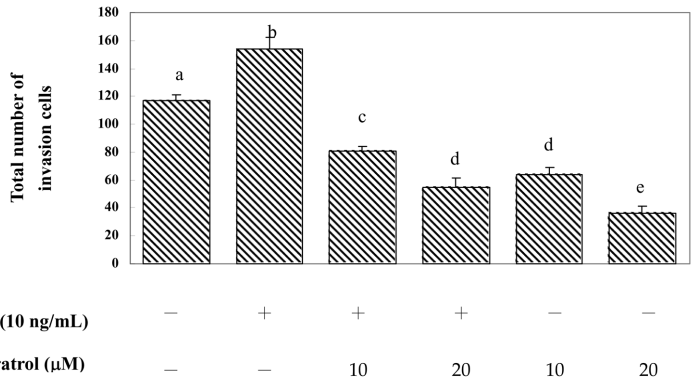


Figure 4. Resveratrol inhibited IGF-1 mediated invasion of human breast-cancer cells. Confluent human breast-cancer cells were cultured in L-15 medium with 10% fetal bovine serum at 37°C. After washing out the media, breast-cancer cells were trypsinized and pre-incubated in L-15 medium with various concentration of resveratrol (0 – 20 μM) for 2 h. The invasion of tumor cells was analyzed in transwell Boyden chambers with a polyvinyl/pyrrolidone-free polycarbonate filter of 8-μm pore size. Each filter was coated with 100 μL of a 1:10 diluted matrigel in cold L-15 medium to form a thin continuous film on the top of the filter. Human breast-cancer cells (50 000 cells/well) stimulated with IGF-1 (10 ng/mL) were added to each of triplicate wells in L-15 medium containing various concentrations of resveratrol (0 – 20 μM). After incubation for 16 h, cells were stained and counted as described above, and the number of cells invading the lower side of the filter was measured as invasive activity. Total number of invasive cells is shown in the figure. The biostatistical analysis is shown. Data analysis from three separate experiments is shown as the mean ± SEM of three independent experiments. Different letters represent statistically significant difference, $p < 0.05$.

3.2 The inhibitory effects of resveratrol on IGF-1-mediated activation of PI-3K/Akt pathways in human breast-cancer cells

As described above, PI-3K signaling pathway plays an important role in IGF-1-mediated migration of human breast-cancer cells. To examine the inhibitory effects of resveratrol on IGF-1-mediated PI-3K/Akt signaling, we measured the phosphorylated Akt by using Western blotting analysis. As shown in the Fig. 2, we found that IGF-1 strongly induced the phosphorylated states of Akt protein within 30 min although constitutive activation of Akt was seen in unstimulated MDA-MB 435 cells.

Resveratrol inhibited the IGF-1-mediated activation of Akt in a dose-dependent manner (0, 10 and 20 μM). At concentration of 10 μM, resveratrol inhibited the activation of Akt around 50%. At concentration of 20 μM, resveratrol effectively inhibited the activation of Akt over 70%. Thus, it is plausible that resveratrol inhibits cell migration of human breast-cancer cells in part through the suppression of PI-3K/Akt signaling pathway.

3.3 Resveratrol inhibited IGF-1-mediated expression of MMP-2 in human breast-cancer cells

Resveratrol, an effective chemoprevention agent, has been shown to potentially block tumor development [24, 29, 30]. In the current study, we examined the possibility whether resveratrol could suppress MMP activity and tumor cell invasion. To examine the effects of resveratrol on cellular invasion, we first measured the MMP expression by zymogram analysis and Western blotting analysis. As shown in Fig. 3A, IGF-1 induced the activity of MMP-2 in human breast-cancer cells. Resveratrol could significantly inhibit the MMP-2 activity in a dose-dependent manner. The results of Western blotting analysis showed that IGF-1 slightly induced the expression of MMP-2 protein (Fig. 3B). No significant difference of MMP-2 gene expression between groups was observed in this study using Q-PCR analysis (data not shown). These results suggest that resveratrol could play a protective role in the prevention of breast-cancer cell invasion via suppressed expression and release of MMP-2 enzyme.

3.4 Resveratrol inhibited IGF-1-mediated invasion of human breast-cancer cells

To further examine the possible role of resveratrol in the prevention of cancer cell invasion, we analyzed the invasion indices by using Boyden chamber coated with matrigel. As shown in Fig. 4, IGF-1 induced cell invasion moderately. Resveratrol could significantly block cell invasive activity in the presence or absence of IGF-1. The increasing level in invasion index is similar to expression of MMP in the presence of IGF-1. These results suggest IGF-1 not only regulates migration of breast-cancer cells but also induces MMP expression during cellular invasion. Resveratrol effectively inhibited IGF-1-mediated migration and invasion of human breast-cancer cells *in vitro*. Thus, resveratrol

could play a protective role in the prevention of tumor cell invasion and metastasis.

4 Discussion

In this study, we demonstrated the involvement of a novel mechanism for resveratrol inhibition of IGF-1-mediated cell migration and invasion in human breast-cancer MDA-MB 435 cells. These findings provide important insights into the molecular mechanisms of grape seed extract in cancer prevention through potential suppression of tumor invasion. Our results indicated that IGF-1 acted as a stimulant for cellular migration of human breast-cancer MDA-MB 435 cells. Under the stimulation of IGF-1, the MI of MDA-MB 435 cells increased over nine fold. Our work also demonstrated that resveratrol significantly inhibited IGF-1-mediated cellular migration. Cell migration plays an important role in neoplastic cell metastasis [31]. The IGF-1R is a ubiquitous and multifunctional tyrosine kinase that has been implicated in ER-positive breast-cancer MCF-7 cell migration [32]. However, ER-negative breast-cancer MDA-MB 435 cells often express low levels of the IGF-1R and fail to respond to IGF-1 with mitogenesis. Thus, there may be differences in the function of IGF-1R in ER-positive breast-cancer MCF-7 cells and in ER-negative breast-cancer MDA-MB 435 cells. Previous studies indicated that anti-IGF-1R strategies effectively reduced the metastatic potential of different ER-negative cell lines [6, 33], suggesting a very important role of IGF-1R in late stages of the disease.

Here, we examined IGF-1/IGF-1R signaling and function in ER-negative breast-cancer cells. It is well known that PI-3K has been identified as the major mediator of the IGF-1R signal in other cellular systems, and its activity was shown to be critical for the IGF-1-induced cell survival, mitogenesis, and translational regulation of protein synthesis [3, 7, 32]. Previous studies indicated that resveratrol displayed estrogen-like agonistic and antagonistic activities in ER-positive human breast-cancer cells [32, 34 – 36]. The major mechanism is in part through the molecular structure similar to ER E2 [34, 35]. Here, we demonstrated the novel effects of resveratrol on cell migration and invasion by using ER-negative human breast-cancer MDA-MB 435 cells as an *in vitro* model. We found that IGF-1 mainly modulated cellular migration of human breast-cancer cells in an ER-independent manner. As shown in this study, resveratrol could effectively inhibit the migration of ER-negative human breast-cancer cells. These results suggest a novel mechanism of resveratrol on ER-negative human breast-cancer MDA-MB 435 cells.

Previous study also indicated that PI-3K and MEK/ERK play crucial roles in ER-negative human breast-cancer MDA-MB 435 cells. To further investigate the molecular mechanisms of migration in MDA-MB 435 cells, we used specific inhibitors against PI-3K and MEK, respectively. We showed that Wortmannin, a specific inhibitor of PI-3K, could effectively suppress IGF-1-mediated migration of ER-negative human breast-cancer MDA-MB 435 cells. However, no inhibitory effects of MEK inhibitor were observed in this study. Thus, the present study showed that IGF-1-induced migration of ER-negative breast-cancer cells required PI-3K activation. These results are different from what has been observed in other ER-positive human breast-cancer cells [32]. Our results suggested that IGF-1/IGF-1R control cell migration through different signaling pathways in ER-negative human breast cancer. IGF-1R could control non-mitogenic processes independent of the ER status, whereas IGF-1R growth-related functions might depend on ER expression in breast-cancer cells.

Furthermore, we also demonstrated the molecular mechanism of resveratrol during IGF-1/PI-3K signaling pathway in human breast-cancer cells. In this study, PI-3K/Akt signaling pathway has been identified as an important role in cell migration. Previous study also demonstrated that Akt plays a crucial role in breast-cancer cell chemotaxis to chemokine during cancer metastasis [37]. Here, we demonstrated that IGF-1 significantly induced Akt activation. Our work further indicated that resveratrol effectively suppressed IGF-1-mediated Akt activation in a dose-dependent manner. It suggested that resveratrol-mediated suppression of cell migration was in part through the inhibition of PI-3K/Akt pathway. Previous study indicated that the IGF-1-mediated MMP-2 expression is also mainly regulated through the PI-3K/Akt pathway [11]. Thus, it is plausible that resveratrol could further suppress invasion and migration of breast-cancer cells in part through blockade of PI-3K/Akt signaling and MMP-2 expression. In this study, our results based on Western blotting findings showed that resveratrol slightly suppressed MMP-2 expression. No significant difference of MMP-2 gene expression between groups was observed in this study (data not shown). However, zymogram analysis indicated that resveratrol significantly inhibited MMP-2 activity in a dose-dependent manner. These results suggest that resveratrol might modulate MMP-2 activity through post-translational regulation of protein synthesis and release instead of the transcriptional regulation of gene expression. In conclusion, it is suggested that resveratrol could suppress cellular invasion of ER-negative breast-cancer cells through the activation and release of MMP-2 protein into surrounding extracellular matrix. Finally, we demonstrated that resveratrol could inhibit breast-cancer cell invasion in a dose-dependent manner in the absence or presence of IGF-1. These results support our major finding that resveratrol inhibited breast-cancer cell migration and invasion in part through the suppression of PI-3K/Akt signaling pathways and MMP-2 activation. In conclusion, our data suggest that grape constituent, resveratrol, could effectively block breast-cancer cell migration and invasion *in vitro*. In this study, we identified important

roles of resveratrol in the suppression of migration and invasion of ER-negative human breast-cancer cell. The molecular mechanism of actions is mediated in part through the suppression of phosphorylation of PI-3K/Akt and inhibition of MMP-2 expression.

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