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Journal of Nutritional Biochemistry 20 (2009) 1013 – 1020
Journal of Nutritional Biochemistry

S-Allylcysteine modulates the expression of E-cadherin and inhibits the malignant progression of human oral cancer☆,☆☆

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Received 31 March 2008; received in revised form 24 September 2008; accepted 30 September 2008

Abstract

Oral cancer is a prevalent type of cancer in Asian countries. Several studies indicated that garlic extracts such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) have anticancer effects. However, the inhibitory effects of water soluble garlic extracts, S-allylcysteine (SAC), on the malignant progression of oral cancer have not been studied well yet. Thus, the purpose of this study was to investigate the inhibitory effects of SAC on the proliferation and progression of human oral squamous cancer CAL-27 cells. In the present study, we demonstrated that SAC dose dependently inhibited the growth of human oral squamous cancer cells. Our results showed that SAC induced the expression of E-cadherin adhesion molecule. Immunocytochemical staining result also revealed that SAC could restore the distribution of E-cadherin molecule on cell membrane. We further demonstrated that SAC stabilized the adherent junction complex of E-cadherin/β-catenin in oral cancer cells. Treatment with the MAPK/MEK specific inhibitor, PD098059, could up-regulate the expression of E-cadherin molecule. Furthermore, SAC significantly inhibited the activation of MAPK/ERK signaling pathway. These findings were associated with the down-regulation of the SLUG repressor protein. In conclusion, our results indicated that SAC effectively inhibited the proliferation, up-regulated the expression of E-cadherin molecule and stabilized the E-cadherin/β-catenin adherent junction complex in human oral squamous cancer cells. The mechanism of action was in part through the suppression of MAPK/ERK signaling pathway and down-regulation of the SLUG repressor protein.

Keywords: S-Allylcysteine; Mitogen-activated protein kinase; E-cadherin; β-catenin; SLUG; Proliferation; Oral cancer

1. Introduction

Oral cancer is one of the most prevalent types of cancer in Asian countries [1]. It is well known that oral carcinoma is characterized by invasion of malignant cells into the underlying connective tissue and by migration of malignant cancer cells to form metastasis at distant sites [2]. During the physiological status of the epithelium, cell adhesion junction complex molecule such as E-cadherin/β-catenin plays crucial roles in cell–cell interactions [3]. E-Cadherin is a transmembrane glycoproteins associated with the cytoskeleton via cytoplasmic proteins including α-catenin, β-catenin and γ-catenin in the epithelium. The β-catenin protein is associated directly with the E-cadherin molecule in the epithelial cells. Therefore, the aberrant expression of the associated β-catenin proteins could disrupt the function of E-cadherin molecule and cell–cell adhesion [4]. In addition to the interaction with E-cadherin molecule, excessive cytoplasmic β-catenin protein could be translocated into the nucleus and modulate the expression of several genes including cyclin D1, matrix metalloproteinase (MMP)-7 and c-Myc [5]. Normal squamous epithelium of the esophagus showed strong E-cadherin/β-catenin expression especially on cell–cell boundaries except in the superficial layer [6]. Epithelial mesenchymal transition (EMT) plays an important

☆ This material is based upon work supported in part by the National Science Council (grant no. 94-2320-B-030-011) and by China Medical University (CMU) (grant no. CMU95-332).
☆☆ Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of China Medical University.
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doi:10.1016/j.jnutbio.2008.09.007

role in tumor prognosis. EMT is known to dismantle cadherin-medicated cell–cell junctions [7]. However, the function of E-cadherin is frequently lost during the development of most types of human cancers, including carcinomas of the oral cavity, breast, colon, prostate, stomach, liver, esophagus, skin, kidney and lung [8,9]. Thus, disruption of E-cadherin-mediated adhesion is considered as a key step in the progression toward the malignant phase of carcinoma [10–12]. Inhibition of the E-cadherin could also lead to the dissembling of cancer cells and invasiveness [13]. In the process of tumor invasion and metastasis, decreased expression of intercellular adhesion E-cadherin molecule also modulates the proliferation of many types of carcinomas [14]. Increased nuclear level of β-catenin protein was observed in malignant carcinoma [6]. Thus, aberrant regulation of β-catenin expression frequently is correlated with malignant transformation [6]. These evidences suggested that functional or structural irregulation of E-cadherin and β-catenin molecules is associated with decreased level of E-cadherin/β-catenin adherent complex in cancer cells.

Transcriptional regulation of E-cadherin is correlated to MAPK/ERK signaling pathway in epithelial cells. Activation of MAPK signaling pathway phosphorylated several transcriptional factors and suppressed the expression of E-cadherin adhesion molecule [15,16]. The expression of E-cadherin is inhibited by either the activated MAPK/ERK or the aberrant β-catenin levels in nucleus [17]. Previous studies indicated that the promoter activity of E-cadherin was enhanced by the suppression of MAPK/ERK signalling pathway [17]. These results suggested that MAPK/ERK signaling pathway may have repressive effects on the expression of E-cadherin protein. The MAPK/ERK pathway has been shown to be the predominant pathway in the tumorgenesis of many types of cancer including oral cancer [18,19]. MAPK/ERK signaling pathway is activated by extracellular stimuli and has uncovered essential roles in the control of gene expression and protein translation, which have an impact on cell growth and morphological change [20]. The SLUG repressor is a major regulator of the EMT during tumor development [21]. Recent studies suggested that overexpression of the SLUG protein is associated with malignant progression of several types of cancer including esophageal adenocarcinoma [22]. Cumulative evidence indicates that activation of MAPK/ERK signaling pathway could up-regulate the expression of the SLUG repressor protein and suppress E-cadherin gene expression [17]. This autoregulation of E-cadherin was mediated by SLUG, β-catenin and ERK pathways and could play crucial roles in tumorgenesis. Therefore, MAPK/ERK signaling pathway and SLUG repressor protein molecule may become possible targets for treatment with anticancer drug or nutritional intervention.

Experimental data demonstrated that garlic extract such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) with anticarcinogenic effects could inhibit the growth of several types of cancer [23–28]. However, currently, the inhibitory effects of SAC on the malignant progression of oral cancer have not been demonstrated yet.

Therefore, in this study, we determined the inhibitory effect of SAC on cell proliferation and malignant progression of human oral squamous cancer CAL-27 cells.

2. Materials and methods

2.1. Reagents and antibodies

S-Allylcysteine (SAC) was purchased from LKT laboratories, Inc. (St. Paul, MN, USA). Anti-β-actin antibody was purchased from Sigma (St Louis, MO, USA). Anti-phosphorylation ERK 1/2, anti-E-cadherin and anti-β-catenin antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Anti-SLUG antibody was purchased from Abcam, Inc. (Cambridge, MA, USA). Human oral squamous cell CAL-27 cell line was obtained from American Type Culture Collection (Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM)/F-12, fetal bovine serum (FBS) and phosphate buffered saline (PBS) were purchased from GIBCO. S-Allylcysteine was dissolved in PBS at a concentration of 400 mM and stored at −20°C. Immediately before the experiment, the stock solution was added to the cell culture medium.

2.2. Cell culture

Briefly, human oral squamous cancer CAL-27 cells were cultured in a 37°C humidified incubator with 5% CO_2 and grown to confluency. Cells used in different experiments have a similar passage number. The DMEM/F-12 medium was supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine and 1.5 g/L sodium bicarbonate. Human oral squamous cancer CAL-27 cells were incubated with different concentrations of SAC for variable time points.

2.3. Assessment of cell viability

MTT assay was conducted to detect the cell viability. Human oral squamous cancer CAL-27 cells were seeded in 24-well plates, each well containing 1×10^5 cells. After 24 h, the culture medium was replaced by medium with SAC. The assay was performed in triplicate for each concentration. During 24 h of incubation, 24-well plate was taken out and fresh 3-[4,5-dimethhylthiaoly]-2,5-diphenyltetrazolium bromide (MTT, final concentration 0.5 mg/ml in PBS) was added to each well.

After 2 h of incubation, the culture media were discarded and 200 μl of acidic isopropanol was added to each well and vibrated to dissolve the depositor. The optical density was measured at 570 nm with a microplate reader.

2.4. Cell–cell adhesion assay

Human oral squamous cancer CAL-27 cells were dissociated by trypsin and washed with 25 mM HEPES in

Hank Balance Salt Solution (HBSS) [29]. The cells were then detached with 2.5 mM EDTA in PBS (5 min, 37°C) and carefully prepared with a single-cell solution. After counting, 10,000 single cells were allowed to aggregate in 25 mM HEPES-buffered HBSS/normal culture medium (1:1) in a 24-well plate, in the presence of vehicle control, EDTA (5 mM) or anti-E-cadherin antibody (20 μg/ml) for 24 h. The extent of aggregation index was determined by phase-contrast microscopy by counting in 10 different fields of aggregated cells. The results were described as the aggregated percentage of the total oral cancer CAL-27 cells.

2.5. Immunoprecipitation and Western blotting analysis

Human oral squamous cancer CAL-27 cells were cultured in 10% FBS culture media in the presence or absence of SAC for various length of time. Cells were lysed at 4°C in a buffer containing 1× PBS, 1% Ipegal CA-630 (Sigma), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS) with 100 μM of phenylmethylsulfonyl fluoride (PMSF), aprotinin and a specific phosphatase inhibitor, sodium orthovanadate. Cell lysates were cleared by centrifugation. Cellular proteins were fractioned on 10% SDS-PAGE, transferred to nitrocellulose membrane and blotted with anti-phosphorylation ERK monoclonal antibody, according to the manufacturer's instructions. The blots were stripped and reprobed with anti-β-actin antibody as loading control. Level of E-cadherin was measured by using the same procedure described above.

2.6. Immunoprecipitation and Western blotting analysis of β-catenin

Human oral squamous cancer CAL-27 cells were cultured in 10% FBS culture media in the presence or absence of SAC for 24 h. The total cells were washed twice with PBS and lysed at 4°C in 0.5 ml of solution containing 50 mM Tris (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 100 mg/ml PMSF. For immunoprecipitation and Western blotting analysis, total cell lysates were immunoprecipitated with anti-E-cadherin primary antibody and blotted with anti-β-catenin antibody.

2.7. Quantum dot-based immunofluorescence and imaging techniques

Human oral squamous cancer CAL-27 cells cultured in DMEM medium with 10% FBS in a tissue culture dish were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. Around 2×10^4 cells were cultured on glass eight-well Tek chamber and treated with various concentrations of SAC for 24 h. At the end of the experiment, cells were fixed in 4% paraformaldehyde in 20 mM of HEPES and 150 mM of NaCl for 20 min, permeabilized in 0.01% Triton X-100 in PBS for 10 min, blocked with 1% bovine serum albumin (BSA)/PBS for 1 h and then incubated at room temperature for 1 h with either anti-E-cadherin primary antibody or anti-SLUG primary antibody at 1:50 in blocking solution. At the end of incubation, cells were washed with PBS and incubated with quantum dot (Q-dot) 525 secondary antibody for another 1 h in 1.5% BSA/PBS. Images were acquired on an Olympus BX-51 microscope using the Olympus DP-71 digital camera and imaging system. Mean integrated fluorescence was measured by accessory software. Similar procedures were repeated from three independent experiments.

2.8. Statistical analysis

The quantitative methodology was used to determine whether there was a difference between experimental sets and control sets of oral squamous cancer cells. In brief, statistical analyses of the differences among triplicate sets of experimental and control conditions were performed using SYSTAT software. Confirmation of difference as being statistically significant requires rejection of the null hypothesis of no difference obtained from replicate sets at the P=.05 level with the one-way ANOVA model. Post hoc test was used to determine differences among different groups. We also performed trend analysis to demonstrate a dose-dependent effect.

3. Results

3.1. Inhibitory effects of SAC on the viability of human oral squamous cancer CAL-27 cells

As shown in Fig. 1, SAC inhibited the cell viability of human oral squamous cancer CAL-27 cells in a dose-dependent manner. At concentrations of 2, 5, 10 and

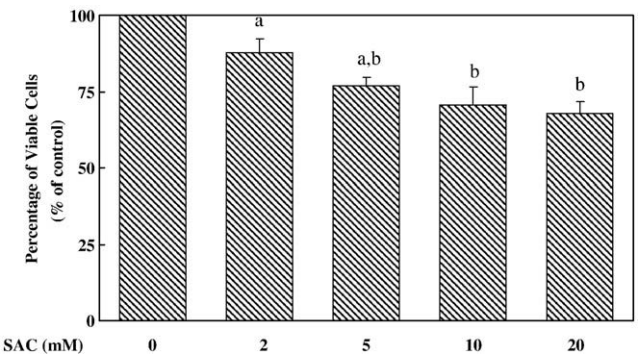

Fig. 1. Inhibitory effects of SAC on the viability of human oral squamous cancer CAL-27 cells. Human oral cancer cells, cultured in DMEM/F-12 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. Approximately 30,000 cells were seeded on each well of a 24-well plate. The human oral cancer cells were then cultured in DMEM/F-12 medium with SAC (0, 2, 5, 10, 20 mM) for 24 h until measurement of cell viability. The incubation was stopped at different time points and measured with MTT assays for cell viability. The analysis of viable cells is described in Materials and Methods. Data from three separate experiments are shown as the mean±S.D. Different letters represent statistically significant difference in pairwise comparisons, P<05.

20 mM, SAC effectively suppressed the viability of human oral squamous cancer CAL-27 cells up to 12%, 23%, 30% and 33%, respectively. These results suggested that SAC could effectively suppress the viability of human oral squamous cancer CAL-27 cells in a dose-dependent manner.

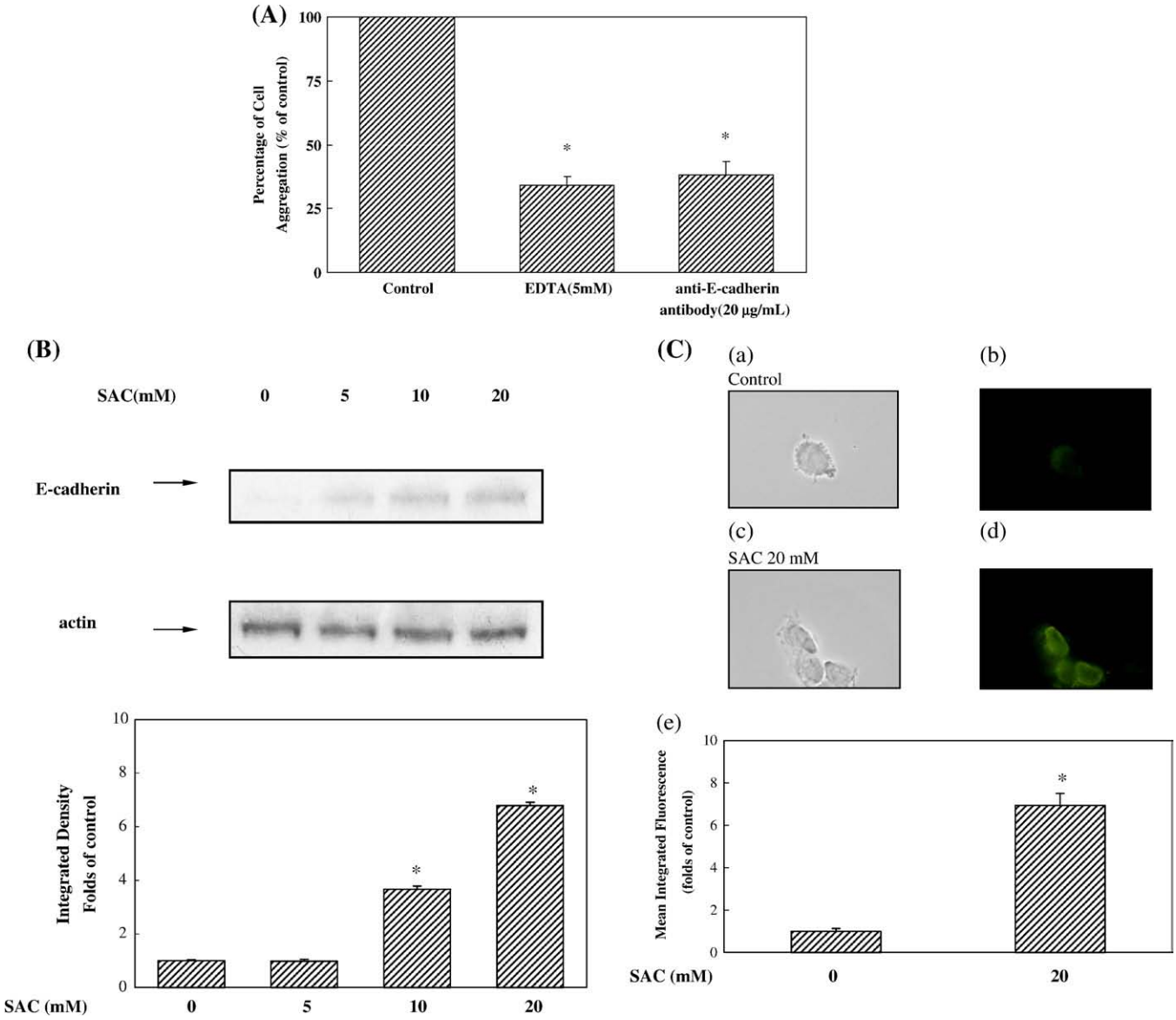

Fig. 2. SAC induced the up-regulation and restoration of the expression of E-cadherin in human oral squamous cancer CAL-27 cells. (A) Human oral cancer cells, cultured in DMEM/F-12 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. For the adhesion assay, approximately 100,000 cells were seeded on each well of a 24-well plate. The human oral cancer cells were then cultured in DMEM/F-12 medium with EDTA (5 mM) or anti-E-cadherin monoclonal antibody (20 μg/ml) for 24 h until the measurement of cellular adhesion. The analysis of adherent cells is described in Materials and Methods. Asterisks represent statistically significant difference compared to the control group, $P<05$. (B) Human oral cancer cells were incubated in DMEM/F-12 medium with 10% FBS in a tissue culture dish with SAC (0, 5, 10, 20 mM) for 24 h. Total cell lysates were blotted with anti-E-cadherin monoclonal antibody as described in Materials and Methods. The levels of detection in cell lysate represent the amount of E-cadherin in human oral cancer cells. The blots were stripped and reprobed with anti-β-actin polyclonal antibody as loading control. Similar results were observed from three independent experiments. The immunoreactive bands are noted with an arrow. Asterisks represent statistically significant difference compared to the control group, $P<05$. (C) Human oral cancer cells, cultured in DMEM/F-12 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. Human oral cancer cells were incubated in DMEM/F-12 medium with 10% FBS in an eight-well Tek chamber with SAC (0, 20 mM) for 24 h. At the end of incubation, cells were blotted with anti-E-cadherin primary antibody and Q-dot 525 secondary antibody as described in Materials and Methods. The levels of detection in cell represent the amount and localization of E-cadherin protein in human oral cancer cells. Images (1000×) were acquired using an Olympus DX-51 fluorescent stereomicroscope and a DP-71 imaging system. (a) and (c) represent images taken in light fields. (b) and (d) represent images taken in dark fields. (e) represents the amount of E-cadherin expression (mean density of integrated fluorescence) in human oral cancer CAL-27 cells. Similar results were observed from three independent experiments. Asterisks represent statistically significant difference compared to the control group, $P<05$.

3.2. SAC Induced the up-regulation and restoration of the expression of E-cadherin in human oral squamous cancer CAL-27 cells

Recent studies demonstrated that E-cadherin plays an important role in the maintenance of cell stability and suppression of cell proliferation. Previous studies indicated that the function of E-cadherin could be blocked by treatment of anti-E-cadherin monoclonal antibody or EDTA [30]. In this study, we also demonstrated that interruption of E-cadherin complex with anti-E-cadherin monoclonal antibody (20 μg/ml) or EDTA (5 mM) would dissemble human oral cancer CAL-27 cancer cells (Fig. 2A). These results suggest that E-cadherin plays an important role in the integrity of human oral squamous cancer CAL-27 cells.

Garlic extracts such as DADS or DATS could induce cell apoptosis in several types of cancer. However, the effects of SAC on EMT and the expression of E-cadherin are still not well known yet. Therefore, we further investigated the effects of SAC on the expression of E-cadherin in human oral squamous cancer cells. As shown in Fig. 2B, SAC induced the expression of E-cadherin molecule in human oral squamous cancer CAL-27 cells in a dose-dependent manner.

To further investigate whether SAC could also stabilize the localization of E-cadherin complex in cell–cell adhesion junction, we analyzed the distribution of E-cadherin molecule by immunocytochemical staining analysis. As shown in Fig. 2C, SAC significantly restored the distribution of E-cadherin in the intercellular junction of human oral squamous cancer cells.

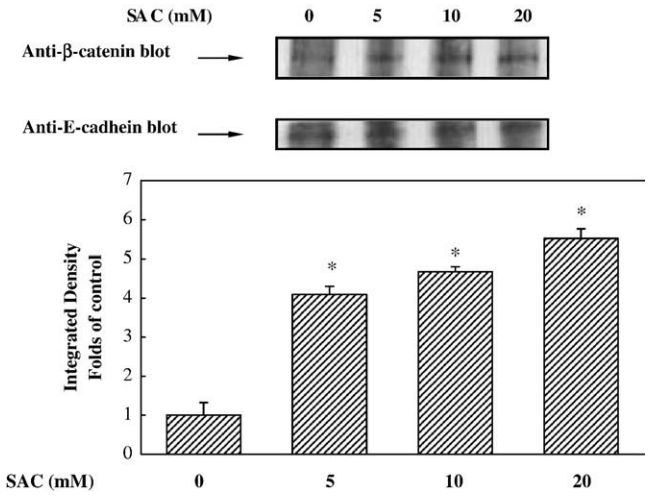

Fig. 3. Effect of SAC on the E-cadherin/β-catenin adherent junction complex of human oral cancer cells. Human oral cancer cells, cultured in DMEM/F-12 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. After washing out the media, human oral cancer cells were incubated in DMEM/F-12 medium with 10% FBS in a tissue culture dish with SAC (0, 5, 10, 20 mM) for 24 h. At the end of incubation, total cell lysates were immunoprecipitated with anti-E-cadherin primary antibody and blotted with anti-β-catenin secondary antibody as described in Materials and Methods. The levels of detection in cell lysate represent the amount and association of β-catenin protein in human oral cancer cells. Similar results were observed from three independent experiments. Asterisks represent statistically significant difference compared to the control group, $P<05$.

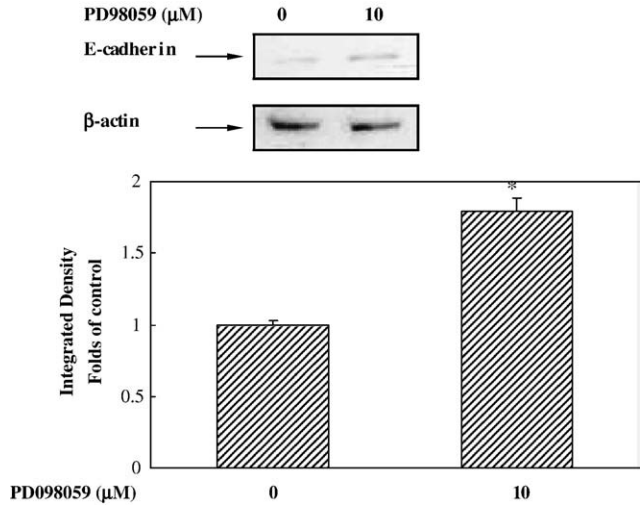

Fig. 4. Inhibition of ERK-1/2 could restore the expression of E-cadherin in human oral squamous cancer CAL-27 cells. Human oral cancer cells, cultured in DMEM/F-12 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. After washing out the media, human oral cancer cells were incubated in DMEM/F-12 medium with 10% FBS in a tissue culture dish with PD098059 (0, 10 μM) for 24 h. Total cell lysates were blotted with anti-E-cadherin antibody as described in Materials and Methods. The levels of detection in cell lysate represent the amount of E-cadherin in human oral cancer cells. The blots were stripped and reprobed with anti-actin antibody as loading control. Similar results were observed from three independent experiments. The immunoreactive bands are noted with an arrow. Asterisks represent statistically significant difference compared to the control group, $P<05$.

It suggested that SAC could up-regulate the expression of E-cadherin and possibly stabilize the intercellular junction complex in human oral squamous cancer cells.

3.3. Effect of SAC on the E-cadherin/β-catenin adherent junction complex of human oral squamous cancer cells

Since loss of E-cadherin and aberrant regulation of β-catenin could lead to the loss of E-cadherin/β-catenin complex and play important roles in cellular proliferation, we further examined the effects of SAC on the E-cadherin/β-catenin adherent complex of human oral squamous cancer cells. Our results showed that SAC significantly stabilized the E-cadherin/β-catenin adherent complex in human oral squamous cancer cells in a dose-dependent manner. As shown in Fig. 3, SAC stabilized and increased the E-cadherin/β-catenin adherent complex. These results suggested that SAC not only could enhance the expression of E-cadherin adhesion molecule but also stabilize the E-cadherin/β-catenin adherent complex in human oral squamous cancer cells. Therefore, SAC could possibly act as an effective compound to prevent and suppress the malignant progression of oral cancer.

3.4. Inhibition of ERK-1/2 could restore the expression of E-cadherin in human oral squamous cancer CAL-27 cells

Although E-cadherin has been demonstrated as an important cell adhesion molecule, the molecular mechanism of SAC in the regulation of E-cadherin molecule in human oral squamous cancer cells has not been demonstrated yet. To determine whether the modulation of cell adhesion E-cadherin molecules in human oral squamous cancer cells could be further regulated by the MAPK/ERK signaling pathway, we analyzed the expression of E-cadherin adhesion molecule under treatment with a specific MAPK/MEK inhibitor, PD098059. As shown in Fig. 4, the MAPK/MEK-specific inhibitor, PD098059, significantly induced the expression of E-cadherin molecule in human oral squamous cancer cells. This finding suggests that inhibition of MAPK/ERK signaling pathway could modulate the expression of E-cadherin molecule in human oral squamous cancer cells.

3.5. Effect of SAC on ERK 1/2 activation in human oral squamous cancer cells

To explore the molecular mechanisms of SAC in the up-regulation of E-cadherin molecule, we investigate the inhibitory effects of SAC on MAPK/ERK signaling pathway. As shown in Fig. 5, SAC could suppress the phosphorylation of ERK 1/2 molecules without any change in total actin protein. It suggests that SAC could enhance the expression of E-cadherin molecule in part through the suppression of MAPK/ERK signaling pathway. Thus, it is

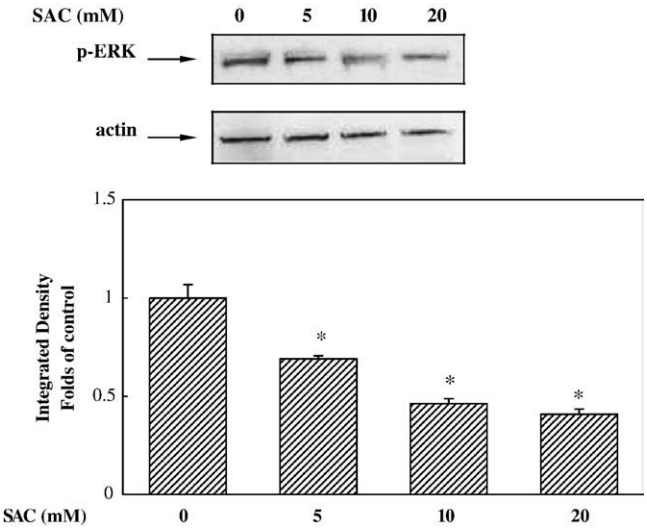

Fig. 5. Effect of SAC on ERK 1/2 activation in human oral squamous cancer cells. Human oral cancer cells, cultured in DMEM/F-12 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. After washing out the media, human oral cancer cells were incubated in DMEM/F-12 medium with 10% FBS in a tissue culture dish with SAC (0, 5, 10, 20 mM) for 24 h. Total cell lysates were blotted with anti-phosphorylation ERK 1/2 antibody as described in Materials and Methods. The levels of detection in cell lysate represent the amount of phoshorylation ERK 1/2 protein in human oral cancer cells. The blots were stripped and reprobed with anti-β-actin antibody as loading control. Similar results were observed from three independent experiments. The immunoreactive bands are noted with an arrow. Asterisks represent statistically significant difference compared to the control group, $P<05$.

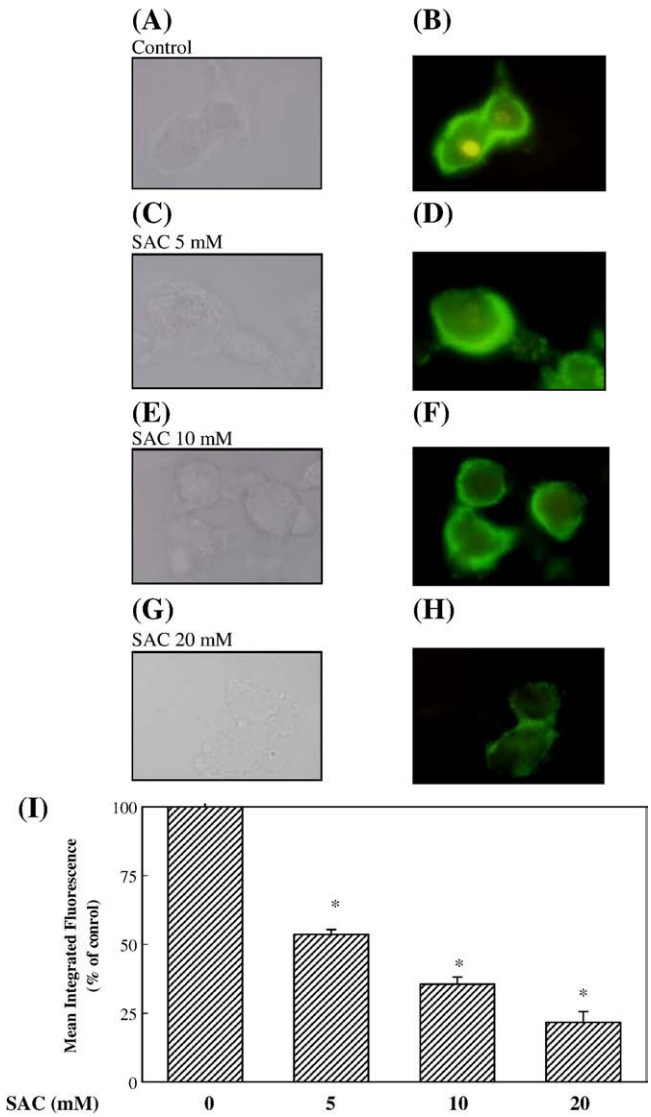

Fig. 6. SAC down-regulates the expression of SLUG transcription factor in human oral squamous cancer cells. Human oral cancer cells, cultured in DMEM/F-12 medium with 10% FBS in a tissue culture dish, were lifted off by trypsinization, pelleted by centrifugation and resuspended in the same medium. Human oral cancer cells were incubated in DMEM/F-12 medium with 10% FBS in an eight-well Tek chamber with SAC (0, 5, 10, 20 mM) for 24 h. At the end of incubation, cells were blotted with anti-SLUG antibody and Q-dot 525 secondary antibody as described in Materials and Methods. The levels of detection in cell represent the amount and expression of SLUG protein in human oral cancer cells. Images (1000×) were acquired using an Olympus DX-51 fluorescent stereomicroscope and a DP-71 imaging system. (a), (c), (e), (g) represent images taken in light fields. (b), (d), (f), (h) represent images taken in dark fields. (i) represents the amount of SLUG expression (mean density of integrated fluorescence) in human oral cancer CAL-27 cells. Similar results were observed from three independent experiments. Asterisks represent statistically significant difference compared to the control group, $P<05$.

plausible that SAC enhanced the expression of E-cadherin molecule and stabilize the adherent junction complex in part through the suppression of MAPK/ERK signaling pathway.

3.6. SAC Down-regulates the expression of SLUG transcription factor in human oral squamous cancer cells

Since we demonstrated that SAC significantly inhibited the MAPK/ERK signaling pathway in human oral squamous cancer CAL-27 cells, we further investigate whether SAC could suppress the accumulation of SLUG repressor protein, a downstream targeted molecule of MAPK/ERK signaling pathway. As shown in Fig. 6, SAC could significantly suppress the expression of SLUG repressor protein in human oral squamous cancer CAL-27 cells in a dose-dependent manner.

SAC significantly inhibited the accumulation of SLUG protein up to 47%, 65% and 78% at concentrations of 5, 10 and 20 mM, respectively. Thus, it is plausible that SAC could effectively suppress the accumulation and expression of SLUG repressor protein in human oral squamous cancer CAL-27 cells. The molecular mechanism of actions is probably through the blockade of MAPK/ERK signaling pathway and suppression of SLUG repressor protein.

4. Discussion

Invasion and metastasis of malignant tumors also require decreased expression of the E-cadherin molecule. Previous studies show that garlic extracts have been identified as strong anticarcinogenic compounds; however, these extracts' molecular mechanism of actions on the expression of E-cadherin has not been investigated yet. In the present study, we found that SAC could effectively suppress the proliferation of human oral squamous cancer CAL-27 cells in a dose-dependent manner. Previous studies indicated that loss of E-cadherin expression is increased in dysplasia of lesions [31]. We further proved that SAC induced the expression of E-cadherin in a dose-dependent manner in human oral squamous cancer cells. We also demonstrated that SAC could restore the distribution of E-cadherin on the surface of human oral squamous cancer cells by using immunocytochemical staining analysis. Our results showed that SAC with anticarcinogenic effect could effectively enhance and restore the distribution of the E-cadherin adhesion molecule in human oral squamous cancer cells. Other studies also suggested that SAC could increase the expression of E-cadherin in human prostate cancer [32]. In consistency with other studies, we found that SAC was able to induce the expression of E-cadherin in human oral squamous cancer cells. Since the E-cadherin and β-catenin complex plays an important role in the stability of cell–cell interaction, we further investigated whether SAC could directly modulate the stability of intracellular adherent complex. Here, we further demonstrated that treatment of SAC could stabilize the E-cadherin/β-catenin adherent complex in human oral squamous cancer cells. These results suggested that β-catenin plays an important role in the regulation of cell-cycle progression and cell proliferation. Increased level of E-cadherin/β-catenin is inversely associated with the proliferation of human oral squamous cancer cells. The possible mechanism is probably through the reduction of nuclear β-catenin protein. Other non–water-soluble garlic extracts such as DADS have been demonstrated to induce cell death in several types of cancer cells. However, we demonstrated that SAC might prevent the EMT process and suppress the transformation of human oral squamous cancer cells in this study. Thus, SAC could be a potent anticarcinoma compound in the prevention of and for therapeutic application in human oral cancer.

Expression of E-cadherin is regulated by a tightly controlled pathway. The E-cadherin promoter consists of Id-1 binding sites and the up-regulation of the Id-1 gene occurs through the transactivation of the promoter by the MAPK/ERK signaling pathway [33]. Several transcriptionally active regulatory elements are also identified in the proximal promoter region, including AP-1, SP-1 and four putative E-boxes [33]. Thus, it is plausible that the MAPK/ERK signaling pathway plays an important role in human oral squamous cancer cells. Our results showed that treatment with the MEK inhibitor, PD098059, could enhance the expression of E-cadherin molecule in human oral squamous cancer cells. It suggested that activation of MAPK/ERK signaling pathway could down-regulate the expression of E-cadherin and induce cell invasion and metastasis. Thus, MAPK/ERK could become an important target signaling pathway in the modulation of E-cadherin expression. Our results showed that SAC could effectively inhibit the activation of MAPK/ERK signaling pathway in human oral cancer cells. Previous studies indicated that activation of Ras oncogene is associated with malignancy and epithelial and mesenchymal transition. Suppression of oncogenic Ras pathway could prevent the tumor prognosis. SAC, a water-soluble garlic compound, can act as modulator to suppress the activation of Ras/MAPK/ERK downstream signaling molecules. Furthermore, the effect of SAC on the decreased activity of MAPK/ERK is in part associated with the up-regulation and restoration of E-cadherin in human oral squamous cancer cells. The reduced activity of the MAPK/ERK signaling pathway is correlated with the down-regulation of SLUG repressor protein.

Taken together; SAC inhibited the MAPK/ERK activities and suppressed the proliferation of human oral squamous cancer cells. SAC enhanced the expression of E-cadherin proteins and increased the stability of E-cadherin/β-catenin adherent complex in human oral squamous cancer cells. Consequently, SAC could further reduce the expression of SLUG repressor protein in human oral squamous cancer cells.

In conclusion, SAC may effectively inhibit the proliferation and malignant progression in human oral squamous cancer cells by suppression of MAPK/ERK signal transduc-

tion pathways and SLUG repressor protein. SAC may be a potent new anticancer compound with improved selectivity toward human oral squamous cancer cells.

Acknowledgment

We thank Miss Yi-Hsuan Shen for assisting with the manuscript preparation.

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