SPANDIDOS PUBLICATIONS

Scutellarin inhibits cell migration by regulating production of $\alpha_v\beta_6$ integrin and E-cadherin in human tongue cancer cells

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Abstract. To examine the effect of scutellarin on adhesion and migration of oral squamous cell carcinoma (OSCC), the HSC-4 and SAS human OSCC cells were treated with various concentrations of scutellarin. Scutellarin cytotoxicity was evaluated by MTT assays; migration of tongue cancer cells was assessed by wound healing and Transwell chemotaxis; $\alpha_v\beta_6$ integrin and E-cadherin expression was assessed by immunofluorescence and reverse transcription polymerase chain reaction. Scutellarin at 75 nM significantly inhibited tongue cancer cell proliferation and at 15 nM, significantly reduced HSC-4 and SAS cell motility by 46.3% and 44%, respectively. Scutellarin inhibited SAS cell adhesion to fibronectin in a dose-dependent manner. However, it had no significant effect on HSC-4 cell adhesion to fibronectin; at the same concentration, HSC-4 cells adhered more strongly to fibronectin than SAS cells. Following treatment with scutellarin, E-cadherin and desmoplakin protein levels were increased, whereas E-cadherin mRNA expression was unchanged; protein levels of $\alpha_v\beta_6$ integrin were decreased-consistent with the change in $\alpha_v\beta_6$ integrin mRNA. After a 3 nM scutellarin treatment, levels of desmoplakin in HSC-4 and SAS cells increased by 79.9% and 74.5%, respectively. Scutellarin (3 nM) increased expression of E-cadherin in HSC-4 and SAS cells by 37.9% and 52%, respectively, and decreased the expression of $\alpha_v\beta_6$ integrin by 45.4% and 47.2%, respectively. This study shows that scutellarin inhibits tumor cell proliferation and migration and regulates cell adhesion in OSCC cells; this may be closely related to up-regulation of E-cadherin and down-regulation of $\alpha_v\beta_6$ integrin.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common head and neck cancer, accounting for over 300,000 new cancer cases worldwide every year (1). Although extensive research is being done on its pathogenesis and management, the 5-year survival rate for OSCC patients has not improved within the last 25 years, remaining at 53% (2). The poor prognosis of OSCC is caused by its local invasion and distant metastasis (3). Thus, metastasis is a major challenge for improved patient outcome.

The metastatic process is comprised of several steps involving cell motility, cell invasion, surface adhesion properties, and degradation of extracellular matrix (ECM) (4). Changes in cell surface adhesion characteristics are reflected in several stages, mediated by a variety of adhesion molecules, including E-cadherin and $\alpha_v\beta_6$ integrin, which control an important mechanism in tumor invasion and metastasis.

Cadherins are transmembrane single-chain glycoproteins, consisting of an extracellular domain, a transmembrane domain and an intracellular domain. They play principle roles in maintaining physical cell-cell adhesion by mediating calcium-dependent homotypic intercellular adhesion in epithelial tissues (5). E-cadherin is expressed in all the living cell layers (6), and is thought to inhibit tumor migration and invasion through the promotion of cell-cell interactions (7). E-cadherin levels are apparently reduced in various tumor types including breast, prostate, pancreas, colon, stomach, bladder, and liver (8-15). In OSCC, reduced E-cadherin expression correlates with less differentiation and invasiveness (16).

Integrins are a family of heterodimeric cell surface receptors that are expressed on most cells; they mediate cell-cell and cell-ECM interactions. Research indicates that integrins regulate diverse processes, including proliferation, migration, cell survival, differentiation, tumor invasion and metastasis, through many signaling pathways (17). Unlike most epithelial integrins, $\alpha_v\beta_6$ integrin is not expressed constitutively by healthy oral epithelia, but is upregulated during tissue remodeling, including remodeling that accompanies wound healing and carcinogenesis (18,19). High $\alpha_v\beta_6$ integrin expression has been described consistently in OSCC, in a

number of studies (18-21). Expression of $\alpha_v ß_6$ integrin was examined in 11 OSCC samples using *in situ* hybridization; ß6 mRNA was detectable in 100% of tumors, although expression did not relate to tumor differentiation, but was maintained in lymph node metastases (21). Expression of $\alpha_v ß_6$ integrin promotes migration and invasion in squamous carcinoma cells (22).

Scutellarin, scutellarin 7-O-ß-D-glucuronide, a known flavone glycoside, is a primary active ingredient in *Scutellaria barbata D. Don* (Lamiaceae). Studies have shown that *Scutellaria barbata* has a variety of biological effects in numerous mammalian systems: free radical scavenging, inhibition of apoptosis, and anti-inflammatory, antitumor, and antimutagenic properties (23,24). Previous studies of our research group have shown that total flavonoids extracted from *Scutellaria barbata* can inhibit SAS cell proliferation, and increase apoptosis *in vitro* (25). Scutellarin, as a flavonoid monomer composition, has been used clinically as an anti-cancer drug (26), but its anti-tumor mechanism is still unknown. To explore the function of scutellarin in cancer growth, we investigated effects of scutellarin on the HSC-4 and SAS human tongue cancer cells in culture for proliferation, adhesion, and migration, and examined effects of scutellarin on protein and mRNA expression of cell adhesion molecules E-cadherin and $\alpha_v ß_6$ integrin.

Materials and methods

Reagents and cells. Scutellarin (purity 99%, HPLC) was purchased from Beidouxing Pharmaceutical Co. Ltd. (Tianjin, China). Human tongue cancer cell lines, SAS and HSC-4 were purchased from the Human Science Research Resources Bank (Osaka, Japan) and maintained at 37˚C in a humidified incubator (Heraeus, Germany) containing 5% CO_2, in Roswell Park Memorial Institute 1640 (RPMI-1640) medium, supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. RPMI-1640 and FBS were obtained from Gibco BRL (Life Technologies, Paisley, Scotland). Bovine fibronectin and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from KeyGen (Nanjing, China). All other reagents and solvents used in experiments were of analytical grade.

MTT cell viability assay. *In vitro* drug sensitivity to scutellarin was assessed by MTT assay. Cells were plated at a density of 10,000 cells in 96-well plates. They were allowed to recover for 12 h and then exposed to different concentrations (3, 15, 75 and 375 nM) of scutellarin for 4, 8, 16, and 24 h. Drug cytotoxicity was evaluated by using a MTT reduction conversion assay. MTT (50 μl) at 5 mg/ml concentration was added to each well; incubation was continued for 4 h. Formazan crystals resulting from mitochondrial enzymatic activity on MTT substrate were solubilized with 150 μl of dimethyl sulfoxide, and absorbance was measured at 490 nm using Stat Fax 2100 microplate reader (Equl-Awaretech, USA). Each combination of cell line and drug concentration was set up in eight replicate wells, and experiments were repeated 3 times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as means ± standard deviation (SD).

Wound healing assay. Cells were detached and prepared in a single cell suspension. Cells ($1x10^4$) were seeded onto a 6-well culture plate and then cultured to at least 95% confluence. Monolayer cells were washed 3 times with phosphate-buffered saline. A cell wound was created with a 200-μl micropipette tip and photographed at a magnification of x100 (Nikon Eclipse TS100, Japan). Monolayer cells with cell wounds were cultured in serum-free medium for another 16 h and then photographed at the same position.

Cell migration assays. Migration of tongue cancer cells were assessed using Millicell® Hanging Cell Culture Inserts (8-μm pore size; Millipore, USA). We added 600-μl RPMI-1640 with 0.4% FBS culture medium to basolateral side of each insert for several minutes to create a chemotactic gradient, then seeded the cells ($1x10^4$) onto the inside of the insert above the membrane. After incubation at 37˚C with 5% CO_2 for 16 h, the medium was discarded and the cells remaining inside the insert were carefully removed. Cells were then fixed with 100% methanol for 3 min at -20˚C. After aspirating the methanol, the cells were stained with Giemsa stain (made in 50% methanol) at room temperature for 30 min. The Giemsa stain was poured off and cells were rinsed with distilled water until excess dye was removed. Cells that had migrated onto the lower surface of the membrane were counted under the microscope at a magnification of x400. Ten visual fields were counted on each filter. Results are expressed as mean (±SD) of the number of cells per visual field.

Adherence assay. A single cell suspension in serum-free medium was prepared by trypsinization of the scutellarin pretreatment cell lines and controls. Cells ($1x10^4$) were plated in fibronectin precoated 96-well culture plates. Each group was examined in 8 replicates. Cells were incubated for 2 h and washed with phosphate-buffered saline (PBS). The number of adherent cells was measured by MTT assay.

Immunofluorescence. SAS and HSC-4 cells were plated on glass coverslips for 24 h in regular growth medium. Following changes of cell culture medium, different concentrations (3, 15, 75 nM) of scutellarin were added for 16 h. For immunofluorescence, coverslips were rinsed in PBS and fixed in 3% paraformaldehyde for 30 min at room temperature and permeated with 0.1% Triton X-100 for 15 min. Cell slides were blocked with normal goat serum (Zhongshan Goldenbridge Biotechnology, Beijing, China) and further incubated with specific mouse anti-human desmoplakin I+II antibody (Abcam Inc., USA; catalog no. ab16434; 1:500 dilution), rabbit anti-human E-cadherin antibody (Zhongshan Goldenbridge Biotechnology; 1:1000 dilution) or mouse anti-integrin $\alpha_v ß_6$ (Millipore; catalog no. MAB2077Z; 1:100 dilution), in an azide-free medium overnight at 4˚C. Slides were subsequently incubated with fluor-conjugated donkey anti-rabbit IgG or fluor-conjugated donkey anti-mouse IgG (Invitrogen, USA) for 1 h. Fluorescence was observed under a fluorescence microscope (Olympus BX51, Japan) equipped with Flash Point software.

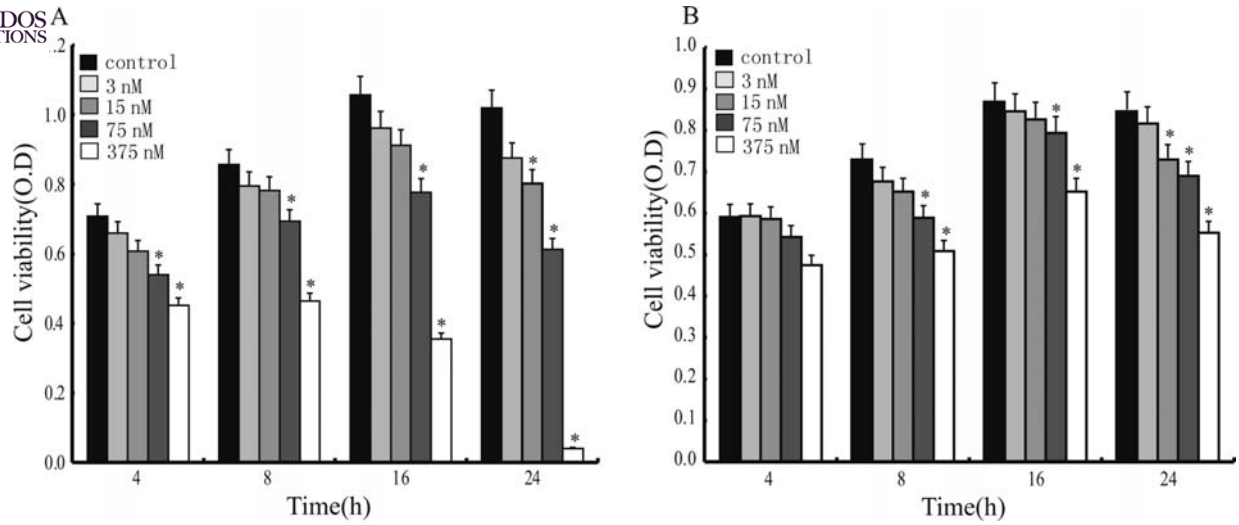


Figure 1. Scutellarin cytotoxicity in HSC-4 and SAS cells. Cells were treated with various concentrations of scutellarin and incubated for 4, 8, 16 and 24 h. Scutellarin cytotoxicity was detected using MTT degradation assay, shown by absorbance at 490 nm. (A) HSC-4; (B) SAS. Data represent the mean ± SEM of three independent experiments. *P<0.05 versus control.

RNA extraction and RT-PCR. RNA was isolated from experimental cells by TRIzol (Invitrogen) according to the manufacturer's protocol. RNA (2 μg) was transcribed into cDNA with a Improm-II™ Reverse Transcription system (Promega, USA) and Oligo-dT primer (Promega). cDNA (2 μl) was added to the PCR system (Bio-Rad, Medein, Mexico) for 30 cycles at annealing temperatures of 43/53/55˚C. $\alpha_v\beta_6$ integrin, E-cadherin and GAPDH-F were amplified using the following primer pairs: 5'-AGAGAAGAAGCAGGCA CATTATC-3' ($\alpha_v\beta_6$ integrin forward), 5'-AGGTAGG ACATCGTTCACAGG-3' ($\alpha_v\beta_6$ integrin reverse); 5'-GCTTC CCTCTTTCATCTCCT-3' (E-cadherin forward), 5'-GACTC CTCCATTCCTTCCAG-3' (E-cadherin reverse); 5'-AACGG ATTTGGTCGTATTGG-3' (GAPDH-F forward), 5'-TGGA AGATGGTGATGGGATT-3' (GAPDH-F reverse). Amplified products were fractionated on 1% agarose gel electrophoresis. The gel was stained with ethidium bromide and photographed under a gel photography system (GE-100, Shanghai, China).

Statistical analysis. Experiments were independently performed at least 3 times. Data are presented as the mean ± SEM (standard error of the mean). Data were analyzed using one way ANOVA. Statistical significance was established at P<0.05.

Results

Scutellarin decreased the viability of tongue cancer cells. To examine the effect of scutellarin on cell viability, viability time curves were created for HSC-4 and SAS cells treated by different concentrations of scutellarin (3, 15, 75 or 375 nM) and incubated for 4, 8, 16, 24 h. Scutellarin cytotoxicity was measured by MTT assay. Result showed that scutellarin inhibited HSC-4 and SAS cell viability significantly in a dose- and time-dependent manner when compared with the DMSO-treated control (Fig. 1A and B). This was particularly marked for cells treated with 75 nM of scutellarin and incubated for 16 h, compared with the control group (P<0.05). We treated cells for 16 h at 3, 15, and 75 nM scutellarin for all subsequent experiments.

Effects of scutellarin on cell migration. Migration is the first step of the more complex process of tumor cell invasion. Using the wound healing assay, we assessed cell migratory activity *in vitro*. As shown in Fig. 2A, wound edges were barely visible in the untreated controls when cultured for 16 h; with increased drug concentration, cell migration was significantly inhibited. HSC-4 cells treated with 15 nM of scutellarin showed almost no migration. SAS cell migration was also significantly inhibited at 15 nM, and completely inhibited by 75 nM scutellarin. We used 24-well Transwell plates (8-μm pore size; Millipore) to validate the results (Fig. 2B). Cells treated with scutellarin showed significantly less migratory activity than untreated cells, in a dose-dependent manner. Scutellarin at 15 nM significantly reduced HSC-4 and SAS cell motility by 46.3% and 44% respectively, in a concentration-dependent manner.

Effects of scutellarin on cell adhesion. We used MTT assay and immunofluorescence chemical methods to test effects of scutellarin on HSC-4 and SAS cell adhesion and adhesion to fibronectin, which is a principal component of ECM surrounding tumor cells. Results showed that scutellarin has different effects on adhesion of HSC-4 and SAS cells to fibronectin (Fig. 3). Scutellarin at 15 nM significantly reduced adhesion of SAS cells to fibronectin by 18.8% in a concentration-dependent manner, while the effect of scutellarin on adhesion of HSC-4 cells to fibronectin had no statistical effect.

We looked for expression of desmoplakin I and II (DP) in HSC-4 and SAS cells treated with various concentrations of scutellarin. Immunocytochemistry showed intensity of expression of desmoplakin in tongue cancer cells was

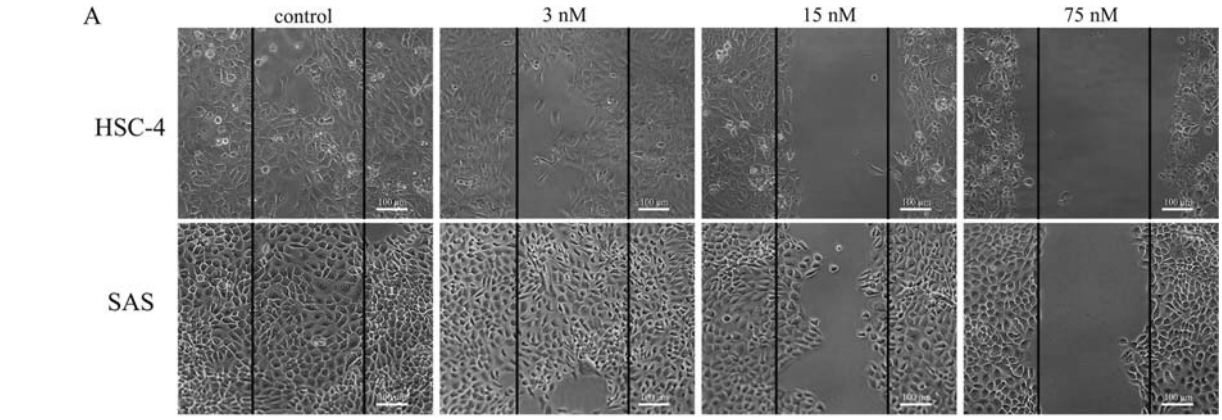


Figure 2. Scutellarin inhibits tongue cancer cell migration. (A) HSC-4 and SAS cells ($1x10^4$) were seeded into 24-well cell culture plates and then cultured to 80% confluence. Cells were scratched with a pipette tip and then pretreated with scutellarin at the indicated dose levels for 16 h, and photographed at a magnification of x100. (B) Effects of scutellarin on tongue cancer cells migratory activity was assessed using Millicell® Hanging Cell Culture Inserts. Following 16 h of incubation with different scutellarin concentrations (3, 15 or 75 nM), migrating cells were stained and counted under a microscope at x400 magnification. The average of 10 fields was recorded in 2-4 replicate studies for each cell line. Values are shown as mean ± standard deviation (SD) of triplicate determinations. *P<0.05 versus control.

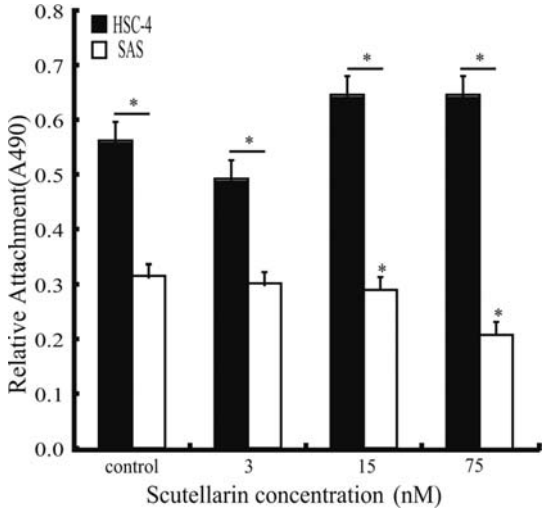


Figure 3. Effects of scutellarin on cell-fibronectin adhesion. Cells were treated with scutellarin at concentrations of 3, 15 or 75 nM for 16 h, and were then subjected to analyses for cell-fibronectin adhesion as described in Materials and methods. Data represent the mean ± SD of at least three independent experiments. Statistical significance was determined by Student's t-test (*P<0.05).

gradually increased, along with the concentration of scutellarin (Fig. 4). After a 3 nM scutellarin treatment, levels of desmoplakin in HSC-4 and SAS cells were increased 79.9% and 74.5%, respectively.

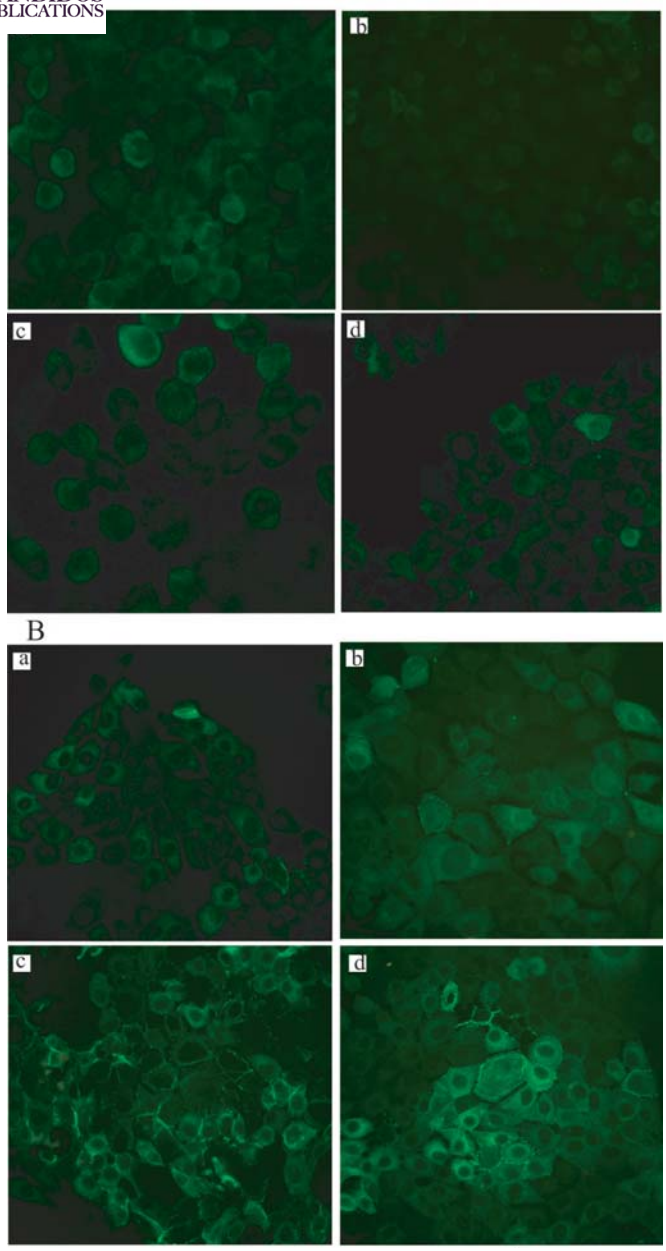
Effects of scutellarin on $\alpha_v\beta_6$ integrin and E-cadherin protein and mRNA expression in tongue cancer cells. We examine the effect of scutellarin on expression of $\alpha_v\beta_6$ integrin and E-cadherin in SAS and HSC-4 cells treated with different concentration of scutellarin (3, 15 and 75 nM). Immunocytochemistry showed that scutellarin increased E-cadherin expression in SAS and HSC-4 cells in a concentration-dependent manner (Fig. 5A and C), but gave no significant change in expression of E-cadherin mRNA (Fig. 6). The results also showed levels of $\alpha_v\beta_6$ integrin and mRNA $\alpha_v\beta_6$ integrin significantly decreased in SAS and HSC-4 cells, along with the concentration of scutellarin (Fig. 5B and D, Fig. 6).

Discussion

Squamous cell carcinoma (SCC) of the tongue is a common malignancy of the oral cavity. Most patients are diagnosed at late stages when tumor metastasis has involved the lymph nodes or distant organs, and surgical treatment alone is not an option. These patients need chemotherapy, radiotherapy and immunotherapy, but these treatments have toxic side effects that affect many patients. It is therefore necessary to find better treatments with fewer adverse effects.

Scutellarin is a major bioactive flavonoid isolated from a Chinese herb, *Herba Scutellariae Barbatae*. It has been shown that extract of *Scutellaria barbata* specifically targets tumor tissue. Recently, its anti-inflammatory and anti-tumor activities have been revealed (26,27).

To study whether scutellarin affects migration and adhesion in tongue squamous cell carcinoma cells, we used MTT



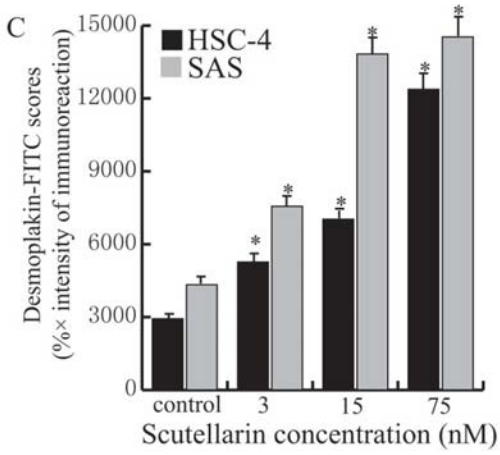


Figure 4. Immunofluorescence staining of desmoplakin in HSC-4 and SAS cells. HSC-4/SAS cell monolayers were incubated beforehand in various concentrations of scutellarin (3, 15 or 75 nM) for 16 h. After fixation, the layers were permeabilized with Triton X-100. In panels A and B, desmoplakin I and II increased along the cell border as scutellarin concentration rose; immunofluorescence was confined to the border of individual cells in the form of spots. All photographs were taken at the same magnification and the images shown are typical of several observations. Image Pro Plus 6.0 (Media Cybernetics Inc., USA) was used to calculate IOD (Integrated Optical Density) from 10 fields for each slide. Mean IOD for desmoplakin in each group were evaluated. C, Data are expressed as mean (± SD). *P<0.01; (A) HSC-4 cells; (B) SAS cells. (a) control, (b) 3 nM, (c) 15 nM, (d) 75 nM.

assays to verify the effect of scutellarin on HSC-4 and SAS cell proliferation. Scutellarin was found to inhibit HSC-4 and SAS cell viability significantly in a dose- and time-dependent manner. The effect was most obvious at 16 h; at this point scutellarin at 15 nM does not show effects on cell proliferation, while scutellarin at 75 nM shows significant cytotoxicity. The results indicate that scutellarin has stronger effects on HSC-4 than SAS cells, suggesting that scutellarin effects differ among various cancer cells, which may be related to the cell degree of differentiation.

We verified whether scutellarin influences HSC-4/SAS cell migration using wound healing assay and transwell chemotaxis experiments. The results showed that, at 15 and 75 nM and after 16 h of treatment, scutellarin can significantly reduce percentages of migrating cells. We can clearly see from Fig. 2A that, after treatment with 15 nM scutellarin, cells with extended pseudopodia were obviously fewer than in the control group, suggesting that scutellarin reduces HSC-4/SAS metastatic potential. This is a phenomenon worthy of further study, on the one hand, suggesting that scutellarin not only induces differentiation and apoptosis, but also has anti-metastasis potential. Scutellarin is a protein kinase C (PKC) inhibitor (28), PKC in mediating cancer cell migration and adhesion. Therefore, we speculated that scutellarin inhibition cell migration may be induced by regulating cell adhesion.

We studied the morphologic effect on adhesion of tongue cancer cells of scutellarin by staining desmoplakin. Results showed that the expression of desmoplakin increased gradually with increased drug concentration. Desmoplakin, a principal desmosomal protein, is present in all desmosome-containing epithelial tissues and functions as a linker protein for cytoskeletal structures. Desmoplakin decreases during SCC progression (30). Down-regulation of desmoplakin expression may represent a useful marker for evaluating risk of distant metastasis formation in oropharyngeal SCC (31). In this study, we found 3 nM scutellarin significant increased desmoplakin expression in HSC-4 and SAS tongue cancer cells. Thus, we speculate that scutellarin enhancement of adhesion between cells may be related to desmoplakin expression.

When we examined the effect of scutellarin on the expression of homogeneous cell adhesion molecule E-cadherin, results showed 3 nM scutellarin significantly increased the expression of E-cadherin. E-cadherin (E-cad) is a key molecule of the adherens junction that connects epithelial cells via homotypic, calcium-dependent interactions. Decreased E-cad expression is generally associated with increased invasiveness and metastasis (8-14). High expression of E-cad in transfected cells is associated with lower invasion through promotion of cell-to-cell adhesion in OSCC cells (16). Loss of E-cad is a significant marker for shortened survival (32). Thus, if we can increase expression of E-cadherin, we might reduce the

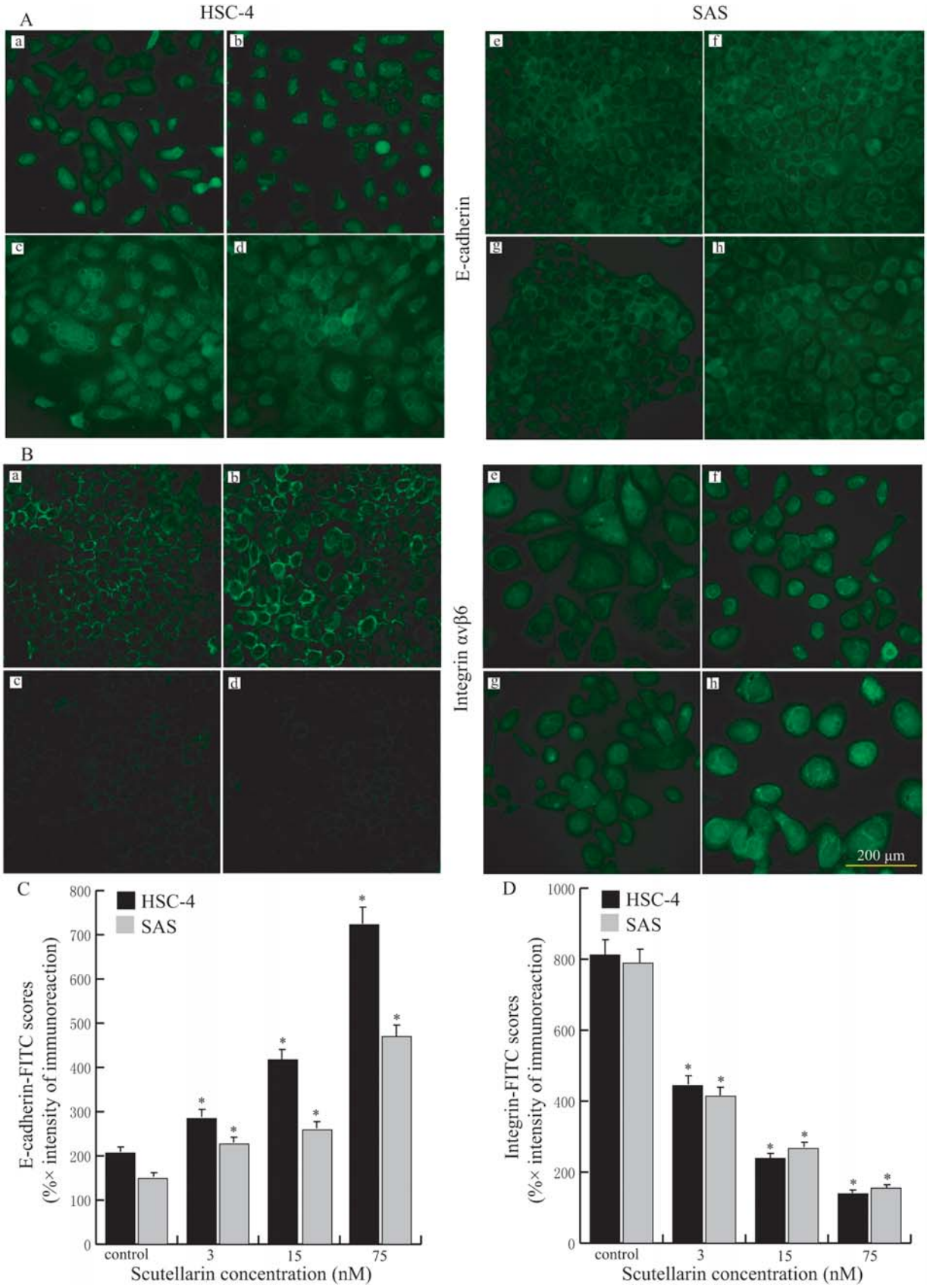


Figure 5. Immunofluorescence staining of E-cadherin and $\alpha_v\beta_6$ integrin in HSC-4 and SAS cells. HSC-4 and SAS cell monolayers were incubated beforehand in various concentrations of scutellarin (3, 15 or 75 nM) for 16 h. After fixation, the layers were permeabilized with Triton X-100. Panels A-a,e and B-a,e represent control staining for E-cadherin and $\alpha_v\beta_6$ integrin. Panels A-b,f and B-b,f represent E-cadherin and $\alpha_v\beta_6$ integrin staining after 3 nM scutellarin treatment. Panels A-c,g and B-c,g represent E-cadherin and $\alpha_v\beta_6$ integrin staining after 15 nM scutellarin treatment. Panels A-d,h and B-d,h represent E-cadherin and $\alpha_v\beta_6$ integrin staining after 75 nM scutellarin treatment. In panels A and C, note significant increases in total amounts of E-cadherin recruited into junctional regions observed in the presence of high concentrations of scutellarin compared to DMSO controls. In panels B and D, expression levels of $\alpha_v\beta_6$ integrin are lower in treated groups than in controls. All photographs were taken at the same magnification and the images shown are typical of several observations. Data are expressed as mean (± SD). Bars, 200 μm. $^*P<0.01$.

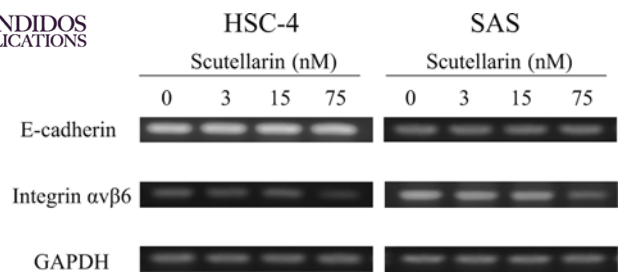


Figure 6. Effects of scutellarin on mRNA levels of $\alpha_v\beta_6$ integrin and E-cadherin in tongue cancer cells. HSC-4 and SAS cells were treated with scutellarin (3, 15, 75 nM) for 16 h for RT-PCR. The mRNA expression levels of E-cadherin and $\alpha_v\beta_6$ integrin were evaluated by RT-PCR. GAPDH was used as an internal control.

number of cells that detach from the primary tumor, thus inhibiting tumor cell invasion and metastasis. In this study, we found that treatment with scutellarin in HSC-4 and SAS cells resulted in dose-dependent up-regulation of E-cad, and improved homotypic intercellular adhesion. We also found that the synthesis of E-cad mRNA was unaffected after treatment with scutellarin. This suggests that scutellarin regulates expression of E-cadherin post-transcriptionally, thereby reducing the invasiveness of cancer cells.

Integrins are a family of heterodimeric cell surface receptors that are expressed on most cells, where they mediate cell-cell and cell-ECM interactions. $\alpha_v\beta_6$ integrin is not expressed constitutively by healthy oral epithelia, but is upregulated during tissue remodeling, including remodeling that accompanies wound healing and carcinogenesis (18,19). Fibronectin is a principal ligand for $\alpha_v\beta_6$ integrin and a major component of the interstitial stroma in invasive oral SCC (33,34). We further verified whether scutellarin influences HSC-4/SAS cell adhesion to fibronectin. The results show that scutellarin inhibits SAS cell adhesion to fibronectin in a dose-dependent manner. Scutellarin is a protein kinase C (PKC) inhibitor (28), and integrin is a substrate of PKC (29). PKC inhibitor could inhibit $\alpha_v\beta_6$ integrin-mediated cell migration (35). The effect of scutellarin adhesion to fibronectin is different in HSC-4 and SAS tongue cancer cells; there is no significant effect on HSC-4 cell adhesion to fibronectin, but at the same concentration, the adhesion of HSC-4 cells to fibronectin is stronger than that of SAS cells. This difference may be prompted by the complexity of scutellarin influence on tumor growth and metastasis.

In the present study, we showed that scutellarin enhances homogeneous adhesion in tongue cancer cells and makes it difficult for cells to break away from the primary tumor to the surrounding tissues. Scutellarin also reduced mobility and ECM adhesion by tongue cancer cells, which may be related to reduction of cell adhesion molecules.

Our group conducted another study showing that scutellarin does not exhibit any significant toxicity on human umbilical vein endothelial cells (data not shown). This suggests that scutellarin is selective between normal and cancer cells. This selectivity of scutellarin to cancer cells may be related to the different genomic stability between cancer and normal cell, but how scutellarin could specifically reduce tumor cell growth without causing toxicity to the normal cellular environment remains unclear. We can speculate that scutellarin acts on unknown cancer-specific genes, thereby permitting a less-than-lethal dose to be used; only cells exhibiting abnormal growth by violating the threshold of the normal cellular environment are affected.

In conclusion, we demonstrated that scutellarin inhibits the growth of tongue cancer cells *in vitro* and has the ability to regulate cell adhesion. Its effectiveness demonstrated here suggests that it may be a promising chemotherapy agent in inhibiting tumor growth and invasion. Further studies are in progress to evaluate the effect of scutellarin on cancer invasion and metastasis *in vivo*.

Acknowledgements

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