Allyl Isothiocyanate Influences Cell Adhesion, Migration and Metalloproteinase Gene Expression in SK-Hep1 Cells

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Allyl isothiocyanate (AITC) has been reported to exhibit antimetastatic activity, but the mechanism remains unclear. The objective of this study was to determine the effect of AITC on cell adhesion, migration, and metalloproteinase gene expression in SK-Hep1 human hepatoma cells. The gene expression profiles of SK-Hep1 cells were obtained by using the HG-U133A Affymetrix GeneChip human genome array containing 14,500 human genes. Twenty antimetastatic genes including COL4A3, ADAMDEC1, CAPN10, CD14, and ITGB1BP3 were over expressed, while the expression of 35 genes such as COL8A1, MYBPC1, ST14, and SOS2 were down-regulated. Semiquantitative RT-PCR confirmed these results in mRNA levels. Based on these *in vitro* results, it can be concluded that AITC might be potentially useful in suppressing tumor cell migration and MMP expression. Exp Biol Med 234:105–111, 2009

Key words: allyl isothiocyanate; metastasis; microarray; SK-Hep1 cell; liver cancer

Introduction

Allyl isothiocyanate (AITC) belongs to the isothiocyanate (ITC) class of chemopreventive compounds in many cruciferous vegetables such as Brussels sprouts, broccoli, cabbage, cauliflower, turnip, radish, and watercress (1). AITC has been shown to induce strong glutathione-*S*-transferase and quinine reductase activities (2, 3). Naturally occurring AITC has also been implicated in the inhibition of B16F-10 melanoma cells-induced metastasis and tumor colony formation by 93% after 21 days of tumor induction in C57BL/6 mice (4); there was also a corresponding increase in the life span of AITC-treated animals.

Malignant cells have the ability to detach from the primary tumor, translocate to distant sites and grow as secondary colonies at the new anatomic locations. This process of the establishment of secondary tumors is known as metastasis. Metastasis is one of the major causes of mortality in cancer patients, and occurs as a complex multi-step process, which involves adhesion of the cancer cells to the basement membrane, invasion through the basement membrane, circulation, extravasation, and migration at a new distant site (5). Interruption of any of these steps, by any agent, has the potential for abrogating malignant spread and could be used as an antimetastatic agent.

The degradation of environmental barriers such as the extracellular matrix (ECM) and basement membrane is an initial step of this process, and several proteolytic enzymes participate in degrading these barriers (6), with matrix metalloproteinases (MMPs) playing a major role. MMPs are a family of zinc-binding endopeptidases that collectively degrade most of the components of ECM, and they have been implicated in cancer invasion and metastasis (7). In particular, MMP-2 and -9 degrade components of the basement membrane and are strongly implicated in the invasion and metastasis of malignant tumors (7, 8). Therefore, inhibition of MMP activity is important in the prevention of early stage carcinogenesis, particularly the tumor promotion process.

The relatively recent development of DNA microarray is capable of profiling gene expression patterns of several hundreds or thousands of genes in a single experiment. They are an ideal tool to study cancer progression and development and to identify new molecular biomarkers for tumor classification and prognosis (9, 10). Gene expression profiling has been already successfully applied to much

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Table 1. Primers Used in Semiquantitative RT-PCR Confirmation of Selected Gene Expression Changes

Gene symbol	Primer	Sequence (5′→3′)	Product size (bp)
COL4A3	Sense	cga gag gct ttg tct tca cc	404
	Antisense	gag aga aat cca gcc gtg ag	
ADAMDEC1	Sense	aga ctt tct tcg ggc aca ga	483
	Antisense	cca gag gga cac ttg gtg tt	
CAPN10	Sense	act tgc aaa gtc cag cct gt	428
	Antisense	tcc tct gct cag ggt ctt tc	
CD14	Sense	ctt gtg agc tgg acg atg aa	677
	Antisense	tag gtc ctc gag cgt cag tt	
ITGB1BP3	Sense	aag acg gct tca aac agt gg	433
	Antisense	cca tag gga aac gct cac at	
PLXNB3	Sense	cag ttc tcc gct gga acc ag	404
	Antisense	ccc aga cag gtg gct tgt at	
ST14	Sense	gct ccc tga agt cct ttg tg	356
	Antisense	agg gag tgg aag gtc agg tt	
COL8A1	Sense	caa gca ctg ggt aag gca ct	490
	Antisense	agc ctg ggt gga cag agt aa	
MYBPC1	Sense	caa agc agc aga gtg cag ag	378
	Antisense	gct ggt tac aat gcc acc tt	
SOS2	Sense	act gcc tgt gga caa aat cc	448
	Antisense	ctg gag ctt cgc tcc tct ta	
ELK1	Sense	cca ccc ttc ctg tac atc gt	421
	Antisense	tgg atg gaa act gga agg ag	
MAGI1	Sense	ggg att tgg ctt tag cct tc	438
	Antisense	cag tcc acg taa gca agc aa	
VAV1	Sense	ggg cat gac ttc cag atg tt	314
	Antisense	agc cgt aga aag ggt cca at	
PCDHGB6	Sense	ggg tca agc gat agt cgt gt	325
	Antisense	cag ggg gca gat ttt cac ta	
MMP16	Sense	atg gca gca caa gca cat ca	804
	Antisense	gca tcg ata cta gga ggc aa	
MMP24	Sense	cag tac atg gag acg cac aa	866
	Antisense	atg gtc acc atg atg tcc ac	
GAPDH	Sense	tgaaggtcggagtcaacggatttg gt	983
	Antisense	catgtgggccatgaggtccaccac	

cancer research (11–14). Mao *et al*., for example, have identified 38 human genes including 21 novel genes and 17 known genes between 20 couple human normal liver tissues and hepatocellular carcinoma (15). Veeriah *et al*. have found that apple extract strongly inhibited growth of HT29 cells and markedly influenced expression of genes encoding xenobiotic enzymes. They found that the up-regulation of the genes such as GSTP1, GSTT2, GSTM2, CHST5, CHST6, and CHST7 and the down-regulation of EPHX1 can all be related to chemoprotection (16). Gene expression profiling has the potential to provide insights to identify those genes' molecular pathways important in disease evolution.

The present study is designed to investigate the molecular mechanism involved in the inhibitory action of AITC in SK-Hep1 cells. Using microarray technology, differentially regulated genes in response to AITC treatment were identified. Furthermore, we investigated whether an increase in mRNA expression in these expression-changed genes is actually accompanied by treatment of AITC using RT-PCR. The antimetastatic activity of AITC by the Matrigel adhesion, invasion assay, migration, and MMP expression system was studied.

Materials and Methods

Cell Culture. SK-Hep1 human hepatoma cells were obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured as monolayer in DEME-media supplemented with 10% fetal bovine serum (FBS, Gibco BRL) and 1% penicillin/streptomycin in a 37°C humidified incubator (Forma Scientific Co.) containing 5% CO_2 and 95% air. At 50% confluence, the cells were then switched to the medium with FBS-free for 1 day before addition of AITC or control. AITC was treated for appropriated incubation time and washed with cool PBS buffer three times before the isolation of total RNA.

RNA Preparation and Affymetrix GeneChip Hybridization. Total RNA was extracted using the Trizol reagent (Life Technologies, Inc., Carlsbad, CA) according to the manufacturer's instructions. Genes expressed in AITC were analyzed on a high-density oligonucleotide microarray (HG-U133A; Affymetrix, Santa Clara, CA) containing 18,400 transcripts. All hybridization experiments were

Table 2. Upregulation of Gene Expression Induced by AITC Treatment of SK-Hep1 Cells

GenBank ID	Affymetrix ID	Gene name	Gene symbol	Fold change (log ratio)
		Cell adhesion		
U02519	216893_s_at	Collagen, type IV, alpha 3	COL4A3	3.87
NM_014479	206134_at	ADAM-like, decysin 1	ADAMDEC1	2.78
NM_023089	21040_at	Calpain 10	CAPN10	2.67
NM_000591	201743_at	CD14 antigen	CD14	2.44
NM_014446	221051_s_at	Integrin beta 1 binding protein 3	ITGB1BP3	2.39
NM_003319	208195_at	Titin	TTN	1.20
M81104	209543_s_at	CD34 antigen	CD34	1.16
NM_014459	205656_at	Protocadherin 17	PCDH17	1.11
AL049430	216114_at	NCK interacting protein with SH3 domain	NCKIPSD	1.10
AF037261	209253_at	Vinexin beta (SH3-containing adaptor molecule-1)	SCAM-1	1.01
		Cell migration		
NM_005393	205957_at	Plexin B3	PLXNB3	1.64
NM_004783	204878_s_at	TAO kinase 2	AOK2	1.17
NM_000426	205116_at	Laminin, alpha 2 (congenital muscular dystrophy)	LAMA2	1.16
U55258	216959_x_at	Neuronal cell adhesion molecule	NRCAM	1.14
		Metalloproteinase		
NM_020249	220287_at	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 9	ADAMTS9	1.22
AI151479	216261_at	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	ITGB3	1.07

performed in triplicate. Target preparation and microarray processing procedures were performed as described in the Affymetrix GeneChip Expression Analysis Manual (Affymetrix, Santa Clara, CA). Briefly, the extracted total RNA was purified with an RNeasy kit (Qiagen, Valencia, CA). Twenty μg of total RNA was used to synthesize double-strand cDNA with SuperScript II reverse transcriptase (Life Technologies, Inc., Rockville, MD) and a T7-(dT)24 primer (Metabion, Germany). Then, biotinylated cRNA was synthesized from the double-strand cDNA using the RNA Transcript Labeling kit (Enzo Life Sciences, Farmingdale, NY) and was purified and fragmented. The fragmented cRNA was hybridized to the oligonucleotide microarray, which was washed and stained with streptavidin-phycoerythrin. Scanning was performed with an Agilent Microarray Scanner (Agilent Technologies, Palo Alto, CA). The data were processed by Affymetrix Microarray Suite 5.0 to derive expression scores. Fold changes were calculated by comparing transcript between control and AITC-treated cells.

Semiquantitative RT-PCR. The results of microarray were verified by the RT-PCR. The primer sets used in this study are listed in Table 1. First-strand cDNA was synthesized with 1 μg of total RNAs and 1 μM oligo(dT15) primer using Omniscript Reverse Transcriptase (Qiagen, Valencia, CA). Using the Taq PCR Master Mix kit (Qiagen), subsequent PCR was performed with 0.5 μL of first-strand cDNA and 20 pmol of primers. The PCR consisted of an initial denaturation at 94°C for 3 mins; 33 three-step cycles at 94°C for 40 secs, 57°C for 40 secs, and 72°C for 1 min; and a final extension at 72°C for 10 mins. The amplified PCR products were loaded onto a 0.8% agarose gel. After ethidium bromide staining, the gel was illuminated on a UV transilluminator and photographed using Polaroid film (Kodak, Needham, MA). The bands' intensities were measured using ImageJ.

Western Blot Analysis. The results of microarray and RT-PCR were verified by the Western blot analysis. Western blot analysis for CD14, SOS2, and VAV-1 expression was performed using an established method. Briefly, cells were washed with ice-cold PBS and harvested with 200 μl of a lysis buffer containing 10 mM Tris-HCl, pH 7.4, 50 mM sodium chloride, 50 mM sodium pyrophosphate, 50 mM sodium fluoride, 100 μM sodium orthovanadate, 2 mM iodoaccetic acid, 5 mM $ZnCl_2$, 1 mM PMSF and 0.5% Triton-X 100. The homogenate was centrifuged at 13,000 rpm for 15 mins at 4°C. Forty micrograms of total protein, as determined by Bio-Rad protein assay, was mixed with 5 × loading buffer and preheated for 5 mins at 95°C. The samples were then loaded on a 10% mini SDS-polyacrylamide gel, and run at 100 V. The proteins were transferred onto PVDF membrane at 100 V/350 mA for 2 hrs using semi-dry transfer apparatus (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked in 5% skim milk in Tris buffered-saline Tween-20 (TBST) solution for 2 hrs at room temperature, then incubated overnight at 4°C with indicated primary antibody (1:1000 dilution). After hybridization with primary antibody, the membrane was washed with TBST three times for 5 mins each, then incubated with secondary antibody for 1 hr at room temperature and washed with TBST three times for 5 mins each. Final detection was performed with enhanced chemiluminescence Western blotting reagents (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Table 3. Downregulation of Gene Expression Induced by AITC Treatment of SK-Hep1 Cells

GenBank ID	Affymetrix ID	Gene name	Gene symbol	Fold change (log ratio)
		Cell adhesion		
BE877796	214587_at	Collagen, type VIII, alpha 1	COL8A1	−4.73
BF593509	214087_s_at	Myosin binding protein C, slow type	MYBPC1	−3.01
BF692958	217576_x_at	Son of sevenless homolog 2 (Drosophila)	SOS2	−2.98
AF000672	210850_s_at	ELK1, member of ETS oncogene family	ELK1	−2.68
NM_004742	206144_at	Membrane associated guanylate kinase	MAGI1	−2.67
NM_005428	206219_s_at	Vav 1 oncogene	VAV1	−2.07
AF135156	221682_s_at	Protocadherin gamma subfamily B, 6	PCDHGB6	−1.84
NM_002207	206009_at	Integrin, alpha 9	ITGA9	−1.79
NM_006113	218807_at	Vav 3 oncogene	VAV3	−1.62
NM_000711	206956_at	Bone gamma-carboxyglutamate (gla) protein (osteocalcin)	BGLAP;PMF1	−1.26
NM_014932	205893_at	Neuroligin 1	NLGN1	−1.2
AI741056	209879_at	Selectin P ligand	SELPLG	−1.18
AW117456	222020_s_at	Neurotrimin	HNT	−1.18
NM_007164	208037_s_at	Mucosal vascular addressin cell adhesion molecule 1	MADCAM1	−1.16
L33477	207149_at	Cadherin 12, type 2 (N-cadherin 2)	CDH12	−1.14
BE251211	202997_s_at	Lysyl oxidase-like 2	LOXL2	−1.12
AF305083	209892_at	Fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)	FUT4	−1.05
NM_024767	220512_at	Deleted in liver cancer 1	DLC1	−1.05
NM_007115	206026_s_at	Tumor necrosis factor, alpha-induced protein 6	TNFAIP6	−1.02
		Cell migration		
NM_020423	205607_s_at	SCY1-like 3 (S. cerevisiae)	SCYL3	−1.49
AK022489	215074_at	Myosin IB	MYO1B	−1.42
NM_005902	205398_s_at	SMAD, mothers against DPP homolog 3 (Drosophila)	SMAD3	−1.18
AI797657	209010_s_at	Triple functional domain (PTPRF interacting)	TRIO	−1.17
U55258	216959_x_at	Neuronal cell adhesion molecule	NRCAM	−1.14
AL161955	216697_at	Triple functional domain (PTPRF interacting)	TRIO	−1.03
		Cell invasion		
U20428	216906_at	Suppression of tumorigenicity 14	ST14	−4.59
		Metalloproteinase		
NM_014479	206134_at	ADAM-like, decysin 1	ADAMDEC1	−2.78
NM_022564	208166_at	Matrix metalloproteinase 16 (membrane-inserted)	MMP16	−1.53
NM_006690	208387_s_at		MMP24	−1.44
NM_025003	220717_at	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 20	ADAMTS20	−1.24
NM_003813	207665_at	A disintegrin and metalloproteinase domain 21	ADAM21	−1.04

Statistical Analysis. All experiments were replicated three times. Mean standard deviation, mean square errors, ANOVA, correlation and interaction of main effects were calculated using Sigmastat computer software 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using the Student-Newman-Keuls Method for multiple comparisons. A $P < 0.05$ was considered statistically significant.

Results

In our previous studies, we determined the effects of AITC on the cell proliferation, adhesion, invasion, migration and matrix metalloproteinase (MMP) expression which were investigated in highly invasive SK-Hep1 human hepatoma cells (17). AITC at 48 hrs treatment significantly reduced cell proliferation in a time- and dose-dependant manner with 20% and 53% by 5 μM and with 10 μM AITC, respectively. The activity of AITC in decreasing Matrigel adhesion, Transwell migration, and MMP expression system was confirmed.

The inhibitory effect of AITC could be mediated through the modulation of gene expression in human hepatocarcinoma cells. The gene expression profiles of SK-Hep1 cells were obtained by using the Affymetrix GeneChip human genome array (HG-U133A) containing 14,500 human genes. These genes were classified according to anti-metastatic function by referring to the literature or using available websites and were categorized into 4 groups as follows: cell adhesion, migration, invasion, and metalloproteinases. Lists of up-regulated (ratio > 2.0) and down-regulated (ratio > 2.0) genes in SK-Hep1 cells treated with AITC are shown in Table 2 and Table 3, respectively. The number of up-regulated antimetastatic activities was 20 genes. The genes including collagen type IV, alpha 3

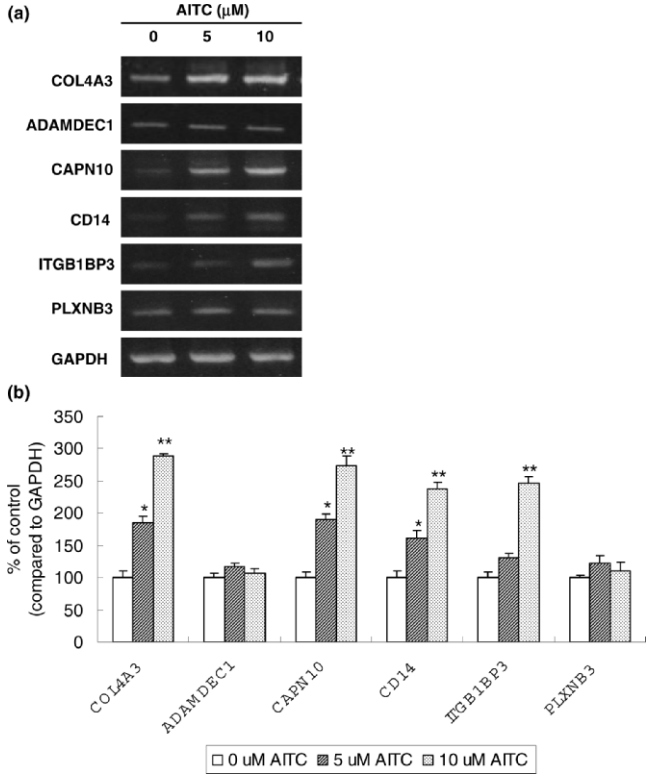

Figure 1. Validation of up-regulated gene expression data after AITC treatment by semi-quantitative RT-PCR in SK-Hep1 cells. The differential expression levels of the selected genes were consistent with the results obtained from microarray analysis. GAPDH was used as an internal control. (a) Representative photos of RT-PCR results. (b) Relative expression values in each gene compared to GAPDH. The intensity of each band was analyzed using a densitometer and results represent three independent experiments. Each bar represents the mean ± SD of three separate experiments. The values marked as * and ** indicate significant differences from other treatments ($P < 0.05$).

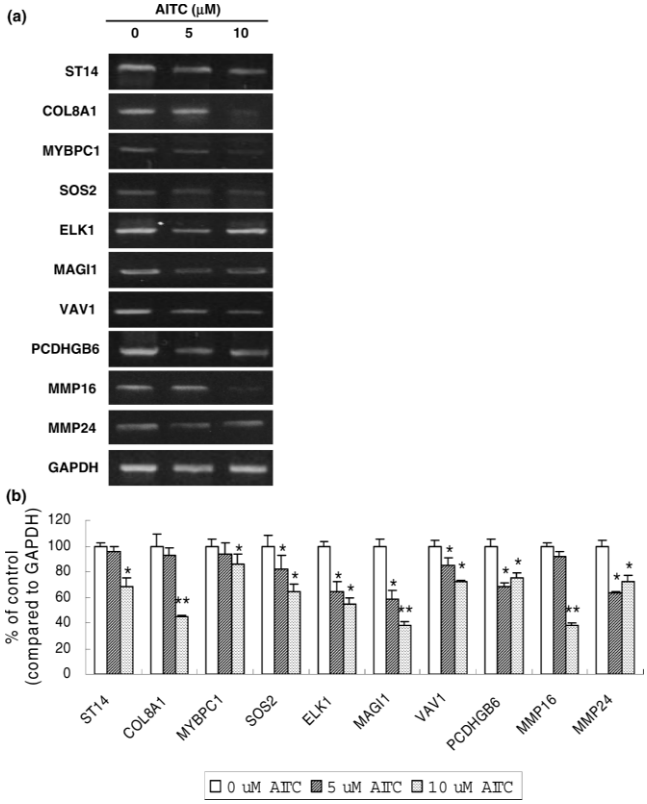

Figure 2. Validation of down-regulated gene expression data after AITC treatment by semi-quantitative RT-PCR in SK-Hep1 cells. The differential expression levels of the selected genes were consistent with the results obtained from microarray analysis. GAPDH was used as an internal control. (a) Representative photos of RT-PCR results. (b) Relative expression values in each gene compared to GAPDH. The intensity of each band was analyzed using a densitometer and results represent three independent experiments. Each bar represents the mean ± SD of three separate experiments. The values marked as * and ** indicate significant differences from other treatments ($P < 0.05$).

(COL4A3), ADAM-like decysin 1 (ADAMDEC1), calpain 10 (CAPN10), CD14 antigen (CD14), and integrin beta 1 binding protein 3 (ITGB1BP3) were over expressed. While the expression of 35 genes such as collagen type VIII alpha 1 (COL8A1), myosin binding protein C (MYBPC1), suppression of tumorigenicity (ST14), and son of sevenless homolog 2 (SOS2) were down-regulated.

To verify the expression of the genes identified in microarray experiments, semiquantitative RT-PCR was performed using the same RNA as that used in microarray analysis. Among the 55 expression changed genes, we tested 14 genes (6 for up-regulation and 8 for down-regulation) with semiquantitative RT-PCR (Figs. 1, 2). We found that the results were in good agreement with those from the microarray data, in that the observed differences were not significant. From the list of up-regulated genes, COL4A3, ADAMDEC1, CAPN10, CD14, ITGB1BP3, PLXNB3, and ST14 were selected. From the list of down-regulated genes, COL8A1, MYBPC1, SOS2, ELK1, MAGI1, VAV1, and ST14 were selected. The RT-PCR results, as shown in Figures 1 and 2, demonstrated identical expression pattern for all selected genes as revealed by microarray analysis. These results showed that the mRNA levels of CAPN10, CD14, ITGB1BP3, PLXNB3, and ST14 were confirmed to be up-regulated. All selected genes for down-regulation in microarray were also down-regulated at 24 hrs after AITC treatment by RT-PCR. In particular, the mRNA of ADAMDEC1 was not increased by AITC; on the other hand, the expression of ELK was decreased at 5 μM AITC but not at 10 μM AITC treatment.

Similarly, Western blotting confirmed CD14, VAV1, and SOS2 protein expression after AITC treatment (Fig. 3). CD14 protein expression was increased in 1.8- and 2.5-fold with 5 and 10 μM AITC treatments, respectively. The VAV1 and SOS2 were down-regulated in protein levels after AITC treatment in a concentration dependent manner (0–10 μM).

Discussion

AITC has been shown to be effective in blocking initiation as well as progression of various chemically

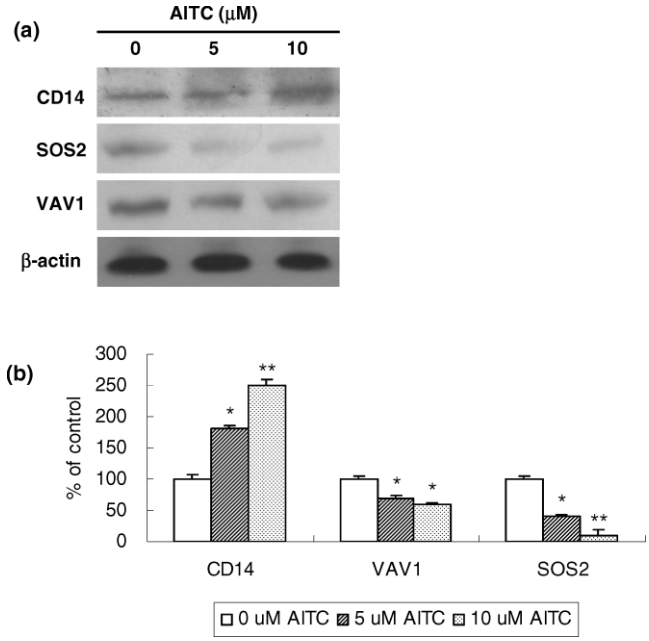

Figure 3. Validation of up- or down-regulated gene expression after AITC treatment by Western blotting analysis in SK-Hep1 cells. (a) Representative photos of Western blot results. (b) Relative expression values in each gene compared to β-actin. The intensity of each band was analyzed using a densitometer and results represent three independent experiments. Each bar represents the mean ± SD of three separate experiments. The values marked as * and ** indicate significant differences from other treatments ($P < 0.05$).

induced carcinogenesis models in animals (18, 19). AITC given by ip injection significantly inhibited the growth of PC-3 human prostate cancer xenografts in mice (18). We previously evaluated *in vitro* the effect of AITC on the proliferation, adhesion, invasion, migration and metastasis of SK-Hep1 cells (17). AITC inhibited SK-Hep1 cell proliferation and caused down-regulation of MMP at both protein and mRNA levels. In particular, MMP-2 and -9 degrade components of the basement membrane and are strongly implicated in the invasion and metastasis of malignant tumors (7, 8). Therefore, inhibition of MMP activity is important in cancer cell metastasis mostly affect cancer development after carcinogenesis.

We studied the expression of genes involved in metastasis after incubation with 10 μM of AITC. The microarray analysis revealed that several targets or pathways might be affected by AITC treatment. Our results show that 55 genes are involved in vital metastatic function of SK-Hep1 cells. The microarray data pointed to an increased expression of the antimetastatic gene such as COL4A3, ADAMDEC, CAPN10, CD14, and ITGB1BP3.

Type IV collagen is the major structural component of glomerular basement membranes (GBM), forming a "chicken wire" meshwork together with laminins, proteoglycans and entactin/nidogen. COL4A3 may induce apoptosis, caspase activation, negative regulation of cancer cell proliferation, and negative regulation of angiogenesis. COL4A3 could be a candidate gene to play a role in mediating the AITC cellular adhesion effects. COL4A3, as belonging to the type IV collagen gene family has well characterized involved in the mediated pathway of metastasis (20).

There is decreased expression of oncogenes, VAV1 and VAV3. Both oncogenes are regulators of cell division and integrin-mediated cell adhesion. There was a down-regulation of ST14 by AITC-treatment. ST14 has known to degrade ECM and take part in tumor invasion and metastasis (21–23). ST 14 gene over-expression increases the local invasion of PKO colorectal cancer cells *in vitro* (23).

Induction of cell-adhesion molecules, which mediate cancer cell adhesion to endothelial cells, would facilitate growth of tumors. Cell adhesion molecules have been demonstrated to play important roles in tumor metastasis (24). Subsequent to cDNA microarray screening, several adhesion molecules that were down-regulated by AITC have been identified, including MAGI-1, MADCAM1, NRCAM, and CDH12. In these genes, MAGI-1 was confirmed to be reduced in mRNA level after AITC treatment. MAGI-1 has been known to play a role as scaffolding protein at cell-cell junctions (25). It is likely that MAGI-1 is involved in tumor cell invasion and the metastasis process by facilitating cell detachment from the primary tumor, migration, and survival in a hostile environment.

We have also identified that the down-regulation of human MYBPC1 correlated with the adhesive ability of SK-Hep1 human hepatoma cell line. MYBPC1 play important roles in cell adhesion, striated muscle contraction, and muscle development (26). Compared to the benign phenotypes the expression of MYBPC-1 was particularly increased in laryngeal squamous cell carcinoma cells, confirmed by Northern hybridization.

Cancer-cell adhesion to endothelial cells mediated by selectins and their ligands has been proposed in cancer metastasis (27). Our results indicated that selectin P ligand (SELPLG) down-regulated compared to control. Selectin-mediated interaction of cancer cell with endothelial cells is known to be implicated in tumor angiogenesis (28).

MMPs consists of over 20 genes that code for proteolytic enzymes, which, in addition to remodeling of the ECM, can affect the processes of cell migration and adhesion. Our results demonstrated that AITC treatment reduced the expression of MMPs 16 and 24 in SK-Hep1 human hepatoma cells. Even though MMP-16/-24 are not the major forms of MMPs, there would be cell-specific expression of MMPs. Using in situ hybridization, Wilson *et al.* (29) demonstrated increased MMP7 expression in tumor epithelial cells in Min mice, whilst MMPs 2, 3, 10, and 13 were up-regulated only in the stromal cells. Taken together, the results clearly demonstrate that up-regulation of several MMP genes occurs during tumor development. Martinez *et al.* (30) have reported that MMPs 10, 13, and 14 were up-regulated in mouse tumor tissues.

The results of this study demonstrated that AITC exhibits antimetastatic activity in SK-Hep1 cells. The anticancer potential of AITC stems from its ability to suppress cancer cell proliferation and invasion by down-regulating the expression of COL8A1 and COL4A3 and inhibiting the activity of MMP-2/-9. However, it might be suggested that AITC-regulated pathways would differ in different cell type. The use of microarray in this study has provided a global view of the cellular response of cancer cells to AITC at genomic levels. This gene expression pattern may bring further insights into the action mechanisms of AITC and provide the information to design more effective anticancer treatments.

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