Allyl isothiocyanate suppressed the proliferation of oral squamous cell carcinoma in vitro, in vivo, and in patient-derived tumor xenografts by downregulating the KDM8/CCNA1 axis

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Abstract

Background: Previous studies have shown that many cruciferous vegetables have anticancer effects, which can be connected with the presence of allyl isothiocyanate (AITC). Histone demethylase KDM8 and cyclin A1 (CCNA1) were required for cell cycle G2/M progression. AITC could induce G2/M arrest of various types of human cancer cells. We aimed to validate KDM8 as a target of the antitumor effects of AITC in patient-derived tumor xenograft (PDTX) models of oral squamous cell carcinoma (OSCC).

Methods: The expression of KDM8 was assessed through tissue microarray (TMA) immunohistochemistry (IHC) assay. The effects of AITC on the expression of KDM8 and cell proliferation were investigated in OSCC cell lines, in PDTX models, and SAS subcutaneous xenograft tumors.

Results: KDM8 was overexpressed in OSCC. AITC repressed the tumor growth of OSCC PDTX and SAS subcutaneous xenograft. Furthermore, AITC downregulated the expression of KDM8 and CCNA1 and induced histone H3K36me2 expression in oral cancer cells.

Conclusions: AITC exerts anticancer effects on oral cancer by inducing cell cycle arrest via inhibiting the KDM8-CCNA1 axis.

Introduction

Oral squamous cell carcinoma (OSCC) is a type of cancer affecting the head and neck, which currently stands as the foremost cause of cancer-related deaths globally. The development of OSCC is frequently linked to various risk factors, including but not limited to smoking, alcohol consumption, chewing habits, and infection with high-risk human papillomavirus [1, 2]. Although surgical procedures, radiation therapy, and chemotherapy have demonstrated significant advantages, the potential for cancer recurrence following treatment may be increased by drug resistance and severe side effects. Therefore, the search for effective and safe drugs is critical. Natural compounds, due to their safety, have recently become a focal point in the quest for anti-cancer drugs as potential alternatives [3].

Aberration of chromatin modifications of histone tails leads to carcinogenesis [4]. Jumonji C domains protein families have been identified as major contributors to various human cancers via epigenetic remodeling [5, 6]. Jumonji-C domain-containing protein 5 (JMJD5), renamed KDM8, is involved in, embryonic development [7], the metabolic regulator [8], osteoclastogenesis [9], circadian rhythm regulation [10], and tumorigenesis [11]. Although the specific ways in which KDM8 contributes to the advancement of cancer are not fully understood, it is hypothesized that its role as a histone demethylase could be significant in controlling the expression of crucial genes implicated in tumorigenesis [4]. KDM8 is highly expressed in various types of cancer such as breast, lung, stomach, prostate, colon, and oral cancers [11–18]. The upregulation of KDM8 in these cancers has been linked with enhanced cell proliferation, migration, and invasion, which indicates its involvement in tumor progression. Additionally, KDM8 can regulate the nuclear translocation of pyruvate kinase muscle isozyme (PKM2) and alter HIF-1alpha-mediated glucose metabolism [8]. In addition, KDM8 functions as a histone demethylase that
specifically removes methyl groups from lysine 36 on histone H3 (H3K36), resulting in the modulation of gene expression. H3K36 methylation is frequently linked with gene activation, and KDM8 has been demonstrated to control genes involved in the cell cycle [4]. KDM8 is also an H3K36me2 histone demethylase that is revealed positively regulate CCNA1 to regulate cancer cell proliferation [4]. The CCNA1 protein functions as a regulatory subunit for cyclin-dependent kinases (CDKs) in the eukaryotic cell cycle. CDK2 is activated by CCNA1 through specific binding, resulting in the phosphorylation of multiple target proteins that facilitate progression through the G1/S and G2/M phases of the cell cycle [19–21]. Furthermore, a research study found that the inhibition of KDM8 can impede metastasis and prompt apoptosis in oral squamous cell carcinoma through regulation of the p53/NF-κB pathway [11]. KDM8 and CCNA1 may have a function in the regulation of the p53 pathway and affect cell cycle progression and DNA damage responses by interacting with p53. Considering these discoveries, KDM8 is a potential target for cancer treatment. In preclinical models, the inhibition of KDM8 activity has been demonstrated to promote programmed cell death in cancer cells and decrease tumor growth [11, 18].

Previous research has shown that natural compounds derived from plants possess chemopreventive, anticancer, and antimetastatic properties and functions [22]. Isothiocyanates (ITCs) are well-established and have been reported to exhibit anticancer effects in human cancers [23–25]. These compounds are present in a variety of cruciferous vegetables, including cauliflower, brussels sprouts, kale, cabbage, horseradish, and wasabi [25, 26]. Allyl isothiocyanate (AITC; 3-isothiocyanato-1-propene, CH2CHCH2NCS) is responsible for the pungent flavor of mustard, horseradish, radish, and wasabi. AITC, which is a sulfur-containing organic compound, is a product of enzymatic hydrolysis of the glucosinolate sinigrin. Research has shown that AITC can hinder cancer cell progression by impeding cell growth, proliferation, migration, and invasion [25, 26]. AITC has been found to regulate DNA methylation, a process that involves the addition of a methyl group to the DNA molecule, and can reduce DNA methylation in cancer cells. This leads to the reactivation of tumor suppressor genes and inhibition of cancer cell growth [27–31]. AITC has also been found to inhibit the activity of histone deacetylases (HDACs), enzymes involved in regulating histone modification [28, 32, 33]. By inhibiting HDAC activity, AITC can increase histone acetylation, alter gene expression, and prevent cancer cell growth [28]. Furthermore, AITC has been shown to induce apoptosis and G2/M phase arrest in human brain malignant glioma GBM 8401 cells [34] as well as apoptotic death in human cisplatin-resistant oral cancer cells [23]. While AITC has been found to repress tumor cell proliferation in various cancer cell lines, including brain, lung, breast, colorectal, bladder, and cervical cancer cell lines [35–37], there is limited research addressing the AITC-mediated effects on OSCC.

Patient-derived tumor xenograft (PDTX) is one of the most promising platforms for simulating human cancer and its complexity. The histopathology of PDTX tumors is very similar to the histopathology of donor lesions. A large amount of evidence, including mutation status, transcriptome, histology, polymorphism, and copy number variation with high fidelity, also supports the view that the PDTX model is very similar to human tumors’ pathophysiology than the traditional cancer-derived xenograft model [38, 39].
In our previous report, we demonstrated a correlation between the high expression of KDM8 and unfavorable prognosis in OSCC [18]. The present study aimed to explore whether AITC, an epigenetic regulator, can modulate KDM8 and potentially regulate gene expression. This modulation could ultimately inhibit oral cancer cell growth in vitro, in vivo, and in PDTX models.

Methods

Chemicals and reagents

The following chemicals and reagents were procured from Sigma-Aldrich (USA): AITC, methylene blue, propidium iodide (PI), ethanol, DMSO, and isopropanol. A concentration of 10 mM AITC was prepared by dissolving it in DMSO and stored at 4°C. RPMI 1640 and fetal bovine serum (FBS) were obtained from Biological Industries (Israel), while RIPA buffer was purchased from Millipore (USA). The present study utilized primary antibodies against KDM8 (AVIVA SYSTEMS BIOLOGY; ARP58120_P050), H3K36me2 (GeneTex; GTX54108), Cyclin A1 (GeneTex; GTX02524), and GAPDH (GeneTex; GTX100118), as well as secondary antibodies (GeneTex; GTX213110-01, GTX213111-01).

Tumor specimens

Samples of tumors and adjacent normal oral mucosa were collected from 26 patients with OSCC at Tri-Service General Hospital of National Defense Medical Center (Taipei, Taiwan, R.O.C.) with their consent and in compliance with Institutional Review Board protocols (TSGH-2-105-05-004). Tumor tissue samples were obtained from archived formalin-fixed, paraffin-embedded (FFPE) specimens collected during diagnosis and stored at hospital pathology departments. The tissue microarray (TMA) procedure was carried out using a method previously described [18].

Immunohistochemistry

The immunohistochemical staining protocol was utilized to evaluate KDM8 antibody binding in all tissue specimens, and the resulting staining scores were determined according to previously published methods [18]. Specifically, the immunostaining score was calculated by multiplying the score for stained tumor cells by the intensity score. The intensity of immune reactivity towards tumor cells was graded on a 0–3 scale, with 0 indicating no staining, 1 indicating weak intensity, 2 indicating moderate intensity, and 3 indicating strong intensity. For each intensity score, the percentage of tumor cells exhibiting nuclear or cytosolic staining was graded on a 5-point scale, with 0 indicating no staining and 4 indicating staining in 75%-100% of cells.

Cell lines and cell culture

The cell lines were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 2 mmol/L L-glutamine, following established protocols [18]. The SCC25 tongue cancer cell line was obtained from the American Type Culture Collection (ATCC), while the SAS
tongue cancer cell line was kindly provided by Dr. Lo from the Institute of Oral Biology, Department of Dentistry, National Yang-Ming University, Taipei, Taiwan.

**In vitro cell proliferation assay**

The antitumor effect of AITC on oral cancer cell growth was performed by the methylene blue dye assay as described previously [40].

**Cell Cycle Analysis**

The study utilized a cell cycle assay method previously described in the literature [41]. Briefly, SAS oral cancer cells were seeded in tissue culture plates at a density of $1 \times 10^6$ cells/dish and subjected to flow cytometry analysis using the FACSCalibur instrument (Becton Dickinson, USA). The cells were treated with different concentrations of AITC (0, 25, and 50 µM) for 24 and 48 hours. Subsequently, the cells were harvested, fixed in chilled ethanol overnight at 4°C, washed with PBS, and resuspended in PBS. The cells were then incubated with 0.5 ml of PI/RNase for 15 min at room temperature.

**Western blot analysis**

A previously published protocol [40] was followed to perform Western blot analysis. In summary, cell pellets were lysed directly in RIPA buffer with added protease and phosphatase inhibitors (Calbiochem, USA). The protein concentration of the resulting supernatants was measured using a BCA protein assay kit (Thermo Scientific, USA). Afterward, 30 µg of cell lysate protein was loaded onto each lane of a 10% SDS-PAGE gel, separated, and transferred onto a polyvinylidifluoride membrane (Amersham, Germany). Finally, specific antibodies were used to probe the membranes for target proteins.

**Establishment of PDTX models and treatment protocol**

The methods used to establish PDTX were outlined in a previous study [41]. PDTX models and treatment protocols were established by ethical guidelines and institutional review board approval. Tumor specimens were obtained from patients with OSCC during their initial surgical treatment and were classified as T4aN2b based on World Health Organization criteria. These tumors were then maintained in RPMI 1640 medium and implanted subcutaneously into nonobese diabetic/severe combined immunodeficiency/gamma (NSG) mice aged 6–10 weeks. The xenograft models were monitored at least twice a week, and tumor volume was calculated using the formula $V = \frac{1}{2} \times (\text{length} \times \text{width}^2)$. Tumor tissues were removed and transplanted serially if the tumor volume reached approximately 3000 mm$^3$. When the tumor volume reached approximately 500 mm$^3$, mice with seventh-generation SC179-PDTXs were randomly assigned to three groups: one receiving AITC (50 mg/Kg/daily, n = 5), one receiving cisplatin (10 mg/Kg/daily, n = 5), and a third group receiving Phosphate buffered saline (PBS, n = 5) as a control. Treatment was administered by intraperitoneal injection for 24 days, and body weight and tumor volume were measured at least twice a week. At the end of the treatment, the mice were sacrificed, and tumors were removed, weighed, and observed. The animal experiments were approved by the National Defense Medical Center Institutional Animal Care and Use Committee, Taipei, Taiwan (IACUC; 16–244 and 18–027).
Mouse SAS xenograft model

Previously described methods [40] were used to perform SAS xenograft animal models. Approval for all experiments was obtained from the National Defense Medical Center Institutional Animal Care and Use Committee, Taipei, Taiwan (IACUC; 16–244). Nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice that were eight weeks old were housed in microisolators under specific pathogen-free conditions. The mice were divided into three groups: the AITC treatment group (n = 5), which received 50 mg/Kg body weight/daily intraperitoneal (i.p.) treatment; the positive control group (n = 5), which received 15 mg/Kg body weight/daily i.p. treatment of 5-Flurouracil (5-FU); and the vehicle control group (n = 6), which received PBS treatment. Each group of mice was subcutaneously injected with $2 \times 10^6$ SAS oral cancer cells. Drug treatments began on the third day after the tumor injection and continued until day 21. Tumor size was monitored at least twice a week, and tumor volume was calculated using the formula $V = \frac{1}{2} \times (\text{length} \times \text{width}^2)$. At the end of the experiment, the mice were sacrificed, and the tumors were excised, weighed, and examined.

UALCAN

UALCAN (http://ualcan.path.uab.edu/index.html) is a web-based tool that provides a thorough examination of gene expression data derived from The Cancer Genome Atlas (TCGA) database [42, 43]. The tool's "TCGA Gene analysis" module was employed to investigate the mRNA levels of KDM8/JMJD5 in head and neck squamous carcinoma (HNSC) patients and healthy individuals. The analysis also explored the correlation of these levels with clinicopathological parameters. The TCGA HNSC dataset, comprising genetic data from 520 individuals, was used for the analysis. The significance of the findings was determined at $P < 0.05$.

Statistical analysis

The statistical analysis of the data was carried out using the software GraphPad Prism (GraphPad Software, San Diego, CA, USA). The statistical significance of the results was determined by performing unpaired, two-tailed Student's $t$-tests. $P < 0.05$ was considered statistically significant.

Results

*KDM8 expression exhibited a significant increase in both human OSCC and cell lines*

In the beginning, we used an immunohistochemical (IHC) stain analysis to determine the expression status of KDM8 protein in 27 OSCC samples and 5 normal oral mucosa samples adjacent to the tumor. Our findings, presented in Table 1 and depicted in Figure 1A, revealed a substantial increase in the levels of KDM8 protein expression in the OSCC tumor samples relative to the normal mucosa samples. To validate our results, we utilized the UALCAN web tool, which utilizes the TCGA database, to analyze the mRNA levels of KDM8 in HNSC tissues and normal tissues. Our analysis, presented in Figure 1C, showed a significant elevation in KDM8 expression levels in HNSC tissues compared to normal tissues ($P<0.05$).
To further support our observations, we performed western blotting analysis on various oral cancer cell lines (SAS, SCC25, and HSC3) and human normal gingival fibroblast cells (HGF) to investigate KDM8 expression. Our results, illustrated in Figure 1D, demonstrated significantly higher levels of KDM8 and CCNA1 expression in the oral cancer cell lines relative to HGF cells.
Table 1. Clinical parameters of the OSCC patients included in this study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=27)</th>
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<tbody>
<tr>
<td><strong>Gender</strong></td>
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<td>T4</td>
<td>9</td>
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<td><strong>Clinical Stage</strong></td>
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<tr>
<td>-II</td>
<td>12</td>
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<td>LN(+)</td>
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<tr>
<td><strong>Death</strong></td>
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*AITC inhibited the growth of oral cancer in preclinical models*
Subsequently, we investigated to determine whether allyl isothiocyanate (AITC) has the potential to impede the proliferation of PDTX in NOD SCID mice. Our findings revealed that when administered at a dose of 50 mg/Kg, AITC exhibited significant anti-tumor activity, similar to that of cisplatin administered at a dose of 10 mg/Kg, in contrast to the PBS-treated control group (as shown in Figure 2A). Additionally, the results demonstrated no apparent toxic effects or noticeable loss of body weight among the different treatment groups (as depicted in Figures 2B and 2C). Furthermore, we evaluated the cytotoxic potential of AITC in vivo by examining its ability to suppress the growth of oral cancer cells SAS xenograft. The results indicated that AITC when administered at a dose of 50 mg/Kg, displayed considerable inhibition of tumor growth, which was comparable to that of 5-FU (administered at a dose of 15 mg/Kg) in oral cancer xenograft (as demonstrated in Figure 3).

**AITC inhibited cell growth and changed the morphology in oral cancer cells**

We investigated the impact of allyl isothiocyanate (AITC), as depicted in Figure 4A, on the cytotoxicity and morphology of oral cancer cells. To assess this, SAS and SCC25 oral cancer cells were exposed to varying concentrations of AITC for 24, 48, and 72 hours (as shown in Figures 4B and 4C). Our findings revealed that AITC exhibited a dose and time-dependent inhibitory effect on the proliferation of tongue cancer cells (as depicted in Figure 4B). After 24 hours of treatment, the IC$_{50}$ of AITC for SAS was approximately 25 μM. Moreover, the cells showed significant morphological alterations, such as shrinkage and rounding, after being exposed to 25 μM of AITC for 24 hours (as shown in Figure 4C).

**AITC induced G2/M cell cycle arrest and apoptosis through modulation of G2/M-associated proteins in oral cancer cells**

The AITC compound was found to induce cell cycle arrest at the G2/M phase, leading to reduced cell proliferation, potentially due to apoptosis as evidenced by the sub-G1 population. This was determined through the examination of cell cycle progression profiles and the percentage of cells in each phase, presented in Figure 5. A significant arrest at the G2/M phase and an increase in apoptotic cells in the sub-G1 groups were observed at a concentration of 25 μM of AITC. Additionally, AITC was observed to affect the levels of proteins linked to the G2/M phase. To confirm the buildup of the G2/M population in SAS oral cancer cells prompted by AITC, the levels of regulated proteins were evaluated. The results demonstrated a significant decline in KDM8 and CCNA1, and an increase in H3K36me2 in SAS cells exposed to AITC, as shown in Figure 6.

**Discussion**

KDM8, a member of an evolutionarily conserved protein family with a JmjC domain, is currently recognized as an oncogene in colon and breast cancer [12, 13, 16, 17]. In this study, we confirmed previous findings that KDM8 is overexpressed in OSCC [11, 18]. KDM8 promotes breast cancer cell proliferation by activating CCNA1, a regulator of the G1/S and G2/M transition [4, 44, 45]. Additionally, KDM8 inhibits p21, a potent inhibitor of a cyclin-dependent kinase, which leads to cell growth retardation,
loss of pluripotency in embryonic stem cells [46], and embryonic lethality when KDM8 is deficient in mouse embryonic cells [47]. These results provide strong evidence that KDM8 is crucial in regulating the G2/M cell cycle.

Previously conducted studies have indicated that allyl isothiocyanate (AITC) can prompt G2/M cell cycle arrest in various types of cancer, such as brain glioma, colorectal adenocarcinoma, and breast adenocarcinoma[34, 48, 49]. CCNA1, which is a member of the highly conserved cyclin family, may be involved in regulating the cell cycle at both G1/S and G2/M transition points [20]. Based on immunohistochemistry-based tests, several studies have suggested that CCNA1 may be linked to poor prognosis in OSCC [50, 51]. In the current study, we discovered that AITC could cause G2/M arrest in OSCC cells and reduce CCNA1 expression. Thus, these results imply that the potential anti-oral cancer effects of AITC may be associated with the suppression of CCNA1.

Histone modification is a crucial mechanism of epigenetic regulation. During post-translational modification (PTM) of histones, histone methylation marks have various effects on transcriptional activation [52]. Several studies have shown that the global regulation of the H3K36me2 histone mark is responsible for epithelial plasticity and metastatic progression [53]. Furthermore, Jumonji domain 2 (JMJD2/KDM4) cluster members are capable of demethylating H3K36me3/2 [52, 54]. These JMJD2 family members are overexpressed in various human cancers [21–23], indicating that H3K36me3 may serve as a tumor suppressor marker. In this study, our findings suggest that the downregulation of KDM8 expression by AITC treatment could induce H3K36me2 expression.

Although AITC has shown promise in its ability to combat cancer, there are still obstacles to its clinical application [23, 55]. PDTX is a type of preclinical cancer model where tumor tissue from a patient is implanted into immunodeficient mice to create a tumor that closely retains the tumor heterogeneity present in the original patient sample, which enables them to replicate the complexity of the tumor, including the tumor microenvironment. This feature is critical for drug development [56]. PDTX models have several advantages for cancer research and drug development, including having good biological relevance, being more predictive of patient response to treatment than cell line models, being suitable for personalized medicine, and having high fidelity. PDTX models can be propagated indefinitely, allowing for long-term studies and drug development. Additionally, PDX models can be used to study tumor evolution and drug resistance, which is difficult to do in patients due to ethical and practical limitations. However, there are also many disadvantages for PDTX models which are high cost and time-consuming, limited engraftment success, and variability in tumor growth rates. PDTX models may exhibit variations in tumor growth rates, which can complicate drug testing standardization. In this study, our results in Fig. 2 have shown that exhibit variations in tumor growth volume and tumor mass. Different cancer types of preclinical PDTX models have been established, however, research on PDTX models for oral cancer is limited [66]. To address this gap, this study aimed to establish PDTX models and verify the anticancer activity of AITC in oral cancer.
In conclusion, AITC inhibited oral cancer growth in vitro, in vivo, and in PDTX models. KDM8 and CCNA1 repression by AITC markedly suppressed oral cancer's proliferation or growth. Thus, AITC might be the candidate adjuvant of OSCC. However, further study should still reveal the mechanism of AITC regulated anticancer effect.

**Declarations**

**Acknowledgments**

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**Author contributions**

CCH conceptualized the study. CCH, CYY, CHT, SLH, and BP performed the biochemical and animal study, analyzed and interpreted data, designed the study, and drafted the manuscript. CKL, CSL, and HYL analyzed the IHC data. HCH and SCC provided the AITC and reagents. GJL helps the animal study and cell cycle analysis. HKS helped designed the study, and interpreted data. WTC and YWC designed the study, interpreted data, and critically revised the manuscript.

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**Availability of data and materials**

All the data and material are available in the Journal of translational medicine website.

**Ethical statement**

This study was approved by the Institutional Review Board (TSGH-2-105-05-004) and performed by the guidelines for the Tri-Service General Hospital of National Defense Medical Center (Taipei, Taiwan,
R.O.C.). All animal experiments were approved by the National Defense Medical Center Institutional Animal Care and Use Committee (Taipei, Taiwan, R.O.C.) (IACUC; 16-244 and 18-027).

Consent for publication

All the authors agree to the publication.

Conflict of interest

The authors have no conflict of interest to declare.

References


**Figures**
Figure 1

High expression of KDM8 existed in oral cancer. (A) Representative samples scored for KDM8 IHC results in normal mucosal and oral cancer tissues are shown. (B) H&E and KDM8 IHC stain for normal tissues adjacent to the tumor (magnification 40x). The score was defined as intensity score x percentage score. Data are expressed as mean ± standard deviation. $P < 0.05$ (Student’s t-test) is represented a significant difference. (C) expression levels of the KDM8 in HNSC patients based on the UALCAN database. (D) KDM8 and CCNA1 protein levels in oral cancer cell lines were determined through western blot analysis. Normal human gingival fibroblast HGF-1 cells were used as the negative control.
Figure 2

AITC inhibited tumor growth in oral cancer PDTX models. (A) In PDTX, NOD/SCID mice bearing patient-derived tumor tissue xenograft were treated with PBS (n = 5), AITC (n = 5; 50 mg/Kg/d), and cisplatin (n = 5; 10 mg/Kg/d). The average tumor weight of each group was compared with that of the control ($P < 0.05$ by Student’s t-test). (B) Changes in tumor volume in oral cancer PDTX models treated for 24 days. Diameters were measured twice a week for 24 days by using Vernier calipers, and the tumor volume was calculated as $1/2 \times L \times W^2$, where W and L are the shortest and longest diameters, respectively. Tumor volumes were compared with those of controls. All data are expressed as mean ± SD. $P < 0.05$ (Student t-test). (C) No significant change was observed in the mice’s body weight compared with that of the vehicle control.
Figure 3

AITC inhibited oral cancer growth in SAS xenograft. (A) *In vivo*, NOD/SCID mice bearing subcutaneous SAS cells were treated with PBS (n = 6), AITC (n = 5; 50 mg/Kg/d), and 5-FU (n = 5; 15 mg/Kg/d). The average tumor weight of each group was compared with that of the control (*P* < 0.05 by Student's *t*-test). (B) Changes in tumor volume in oral cancer SAS xenograft model, which was treated for 21 days. Diameters were measured twice a week for 21 days by using Vernier calipers, and the tumor volume was calculated as $1/2 \times L \times W^2$, where W and L are the shortest and longest diameters, respectively. Tumor volumes were compared with those of controls. All data are expressed as mean ± SD. *P* < 0.05 (Student *t*-test). (C) No significant change was observed in the mice's body weight compared with that of the vehicle control.
Figure 4

Effects of AITC on proliferation in SAS and SCC25 cells. (A) structure of AITC. (B) Cells were treated with various concentrations of AITC for 24, 48, and 72 h, and then cell viability was determined by the methylene blue assay. (C) Representative photographs were captured with 40x magnification of SAS (control and after treatment with AITC for 24 h).
Figure 5

AITC induced oral cancer G2/M arrest.

Flow cytometric cell cycle analysis of SAS cells treated with varying concentrations of AITC (25–50 μM) or DMSO (1 μL/mL) for 24 and 48h. Graphs show cell cycle distribution (A) and distribution quantification percentage (B).
Figure 6

AIrC downregulated KDM8 and CCNA1 expression in oral cancer cells.

Western blot analysis and densitometry for KDM8, H3K36me2, and CCNA1 after SAS cells treated with AITC for 24 h and 48 h.