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ABOUT COVER

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ORIGINAL ARTICLE

Basic Study

Aldehyde dehydrogenase 2 family member repression promotes colorectal cancer progression by JNK/p38 MAPK pathways-mediated apoptosis and DNA damage

Miao Yu, Qian Chen, Yi-Ping Lu

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Abstract

BACKGROUND

Aldehyde (ALDH2) dysfunction has been verified to contribute to human cancers.

AIM

To investigate the molecular mechanism and biological function of ALDH2 in colorectal cancer (CRC) progression.

METHODS

Human CRC cells with high expression of ALDH2 were screened. After shRNA ALDH2 (sh-ALDH2) transfection, phenotypes [proliferation, apoptosis, acetaldehyde (ACE) accumulation, DNA damage] of CRC cells were verified using cell counting kit-8, flow cytometry, ACE assay, and comet assays. Western blotting was used for evaluation of the apoptosis proteins (Bax and Bcl-2) and JNK/p38 MAPK pathway-associated proteins. We subjected CVT-10216 (a selective ALDH2 inhibitor) to nude mice for establishment of SK-CO-1 mouse xenograft model and observed the occurrence of CRC.

RESULTS

The inhibition of ALDH2 could promote the malignant structures of CRC cells, including apoptosis, ACE level, and DNA damage, and cell proliferation was decreased in the sh-ALDH2 group, whereas ALDH2 agonist Alda-1 reversed features. ALDH2 repression can cause ACE accumulation, whereas ACE enhanced CRC cell features related to increased DNA damage. Additionally, ALDH2 repression led to JNK/P38 MAPK activation, and apoptosis, ACE accumulation, and DNA damage were inhibited after p38 MAPK inhibitor

SB203580 and JNK inhibitor SP600125 addition. ACE accumulation and raised DNA damage were recognized in CVT-10216 treated-mouse tumor tissues *in vivo*.

CONCLUSION
The repression of ALDH2 led to ACE accumulation, inducing cell apoptosis and DNA damage by the JNK/p38 MAPK signaling pathway activation in CRC.

Key Words: Aldehyde dehydrogenase 2 family member; Colorectal cancer; Acetaldehyde; Apoptosis; DNA damage; JNK/p38 MAPK

Core Tip: This work demonstrated that a (ALDH2) repression caused the accumulation of acetaldehyde, inducing cell apoptosis and DNA damage by means of activating the JNK/p38 MAPK signaling pathway in colorectal cancer (CRC). ALDH2 is utilized as a therapeutic target for reversing patients with CRC.

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INTRODUCTION

Colorectal cancer (CRC) is a common cancer, showing a high mortality throughout the world[1]. According to the Global Cancer Observatory report in 2020, over 1.9 million new CRC cases and 930000 deaths were estimated[2]. CRC has many risk factors, including environmental and inherited. And Fewer than 10% of patients have an indeed inherited predisposition to CRC[3]. Literatures show that many lifestyle-related factors associate with CRC, including obesity, physical activity, smoking, alcohol intake, and certain dietary variables. Other risk factors, such as being older, whether we have a history of adenomatous polyps (adenomas), personal history of inflammatory bowel disease, and family history of CRC or adenomas, are also risks that we cannot change[4]. Literature also shows that alcohol abuse is an essential risk factor for CRC[5]. Ethanol is mainly oxidized to acetaldehyde (ACE) through ethanol dehydrogenase, and ACE is a reagent that can trigger tumors, including CRC[6,7].

ACE is formed by the ethanol metabolism by ethanol dehydrogenase, catalase, and cytochrome P450 2E1 (CYP2E1). The previous study has shown that ACE can interfere with the antioxidant defense system and produce reactive oxygen species to inhibit DNA methylation and repair and form DNA and protein adducts[6]. The main mitochondrial enzyme that protects cells from ACE toxicity is Aldehyde (ALDH2)[8]. ALDH2 has 19 subtypes, and ALDH2 can detoxify ACE produced by ethanol metabolism in the liver[9]. In several tumor types, ALDH2 inhibition is related to cytotoxicity inhibition, DNA damage, and carcinogenic effects[10,11]. In addition, chromosomal instability helps cancer metastasis through cytoplasmic DNA produced by gDNA cleavage[12]. Furthermore, ALDH2 can inhibit cell migration and proliferation, help apoptosis, and change the epithelial-mesenchymal transition process[13].

We examined the ALDH2 function in CRC and identified that ALDH2 repression can cause raised malignant features. Proliferation capacity is measured by ACE accumulation. ALDH2 repression caused ACE accumulation, which induces DNA damage and cell apoptosis by the JNK/p38 MAPK signaling pathway activation in CRC.

MATERIALS AND METHODS

Cell culture and transfection

Normal human colon mucosal epithelial cell line (NCM460), human CRC cell lines, NCM460, CL-40, SK-CO-1, SW-403, HT-29, COLO-678, and SW480 were purchased from American Type Culture Collection (Manassas, VA, United States). These cells were cultured in Iscove's Modified Dulbecco's Media and added with 10% fetal bovine serum (10099158, ThermoFisher, United States) and antibiotics (1%). The culture environment was 37 °C under 5% CO_2. Cells were preserved with Alda-1 (1 μmol) or with a vehicle for 48 h at 37 °C. The plasmids (RiboBio, Beijing, China) of shRNA oligonucleotides targeting ALDH2 [shRNA ALDH2 (sh-ALDH2): *5′-ATGTCTCCGGTATTATGCC-3′*), and NC (sh-NC: *5′-ACTACCGTTGTTATAGGTG-3′*) were used. These above mentioned plasmids were transfected into CRC cells with Lipofectamine 3000 (L3000150, Invitrogen, United States) and cultured for 2 d.

Quantitative reverse transcriptase PCR

cDNA synthesis from transfected cells was done using total RNA (500 ng) extracted by EcoDry Reverse Transcription

Table 1 The primer sequence for the quantitative reverse transcriptase PCR

Name	Sequence	
ALDH2	Forward	5′-CCTCGGCTACATCAACACG-3′
	Reverse	5′-CCCAACAACCTCCTCTATGG-3′
GAPDH	Forward	5′-GGACCTGACCTGCCGTCTAG-3′
	Reverse	5′-GTAGCCCAGGATGCCCTTGA-3′

Premix (639278, TaKaRa, Tokyo, Japan). Quantitative reverse transcriptase PCR (qRT-PCR) was done by means of SYBR-green (11784200, Invitrogen). The relative expression was calculated through the $2^{-\Delta\Delta CT}$ approach[14] with GAPDH serving as internal reference. Primers are listed in Table 1.

Western blotting assay

The transfected CRC cells were dissolved. Then, the total protein (40 μg) was purified and quantified through Pierce™ BCA protein assay kit (23227, ThermoFisher, United States). After that, we detached proteins by SDS-PAGE (10%) and then shifted them to PVDF membranes (IPVH00010, Millipore, United States). We blocked with 5% skimmed milk (232100, BD, United States) and cultured proteins with anti-ALDH2 (1:1000, ab227021, Abcam, United Kingdom), anti-Bax (1:1000, ab182733), anti-Bcl2 (1:1000, ab182858), anti-γH2AX (1:1000, ab243906), anti-p-JNK (phospho T183+Y185) (1:1000, ab307802), anti-JNK (1:1000, ab208035), anti-p-P38 MAPK (1:1000, ab39398), anti-P38 MAPK (1:1000, ab308333), and anti-β-actin (1:1000, ab8227) 24 h at 4 °C. Proteins continued to incubate with the anti-rabbit secondary antibody (1:5000; SA00001-2, SanYing, China) for one hour after washing the primary antibodies. We examined protein bands by the ECL chemiluminescent system (Thermo Fisher Scientific, United States). Image J was applied for the quantification of protein blots.

Cell counting kit-8 assay

The proliferation capabilities of the transfected CRC cells were analyzed by cell counting kit-8 (CCK-8) assay. We seeded the sh-ALDH2-transfected cells (1×10^3/well) in a 96-well plate. After 1 d, we added CCK-8 reagent (10 μL, Catalog No. AD10, Dojindo Molecular Technologies, Inc., Kumamoto, Japan) to wells at room temperature. At 450 nm, we monitored absorbance at 0, 24, 48, 72, and 96 h for the evaluation of the cell viability.

Flow cytometry analysis

The CRC cell apoptosis was detected using a flow cytometer (LSRII, BD Biosciences, United States). Briefly, cells were harvested by trypsinization and resuspended in 1 × buffer (Annexin V-FITC/PI apoptosis detection kit; SY0471, Beyotime Biotechnology, China). In total, 100 μL of this cell suspension (1×10^6 cells) was incubated with 5 μL Annexin V-FITC and propidium iodide at 4 ˚C in the dark for 15 min. The stained cells were analyzed using a BD FACSCalibur™ flow cytometer and FlowJo software (version 7.2.4; FlowJo LLC). Q2 (early apoptosis) and Q3 (late apoptosis) quadrants' cells were considered as the apoptotic cells.

ACE assay

Methanol (80%, R40121, Thermo Fisher Scientific, United States) was used as an extraction reagent. For sample detection, 800 μL acetonitrile (80%, 4340863, Thermo Fisher Scientific) and dinitrophenylhydrazine (200 μL, D199303, SigmaAldrich, United States) were added. The samples underwent a triple homogenization step, employing the Bertin Precellys 24 Dual Multifunctional sample homogenizer (Bertin, France) at 5500 rpm for 20 s each. Following homogenization, the samples were subjected to a sequential temperature treatment, initially stored at -80 °C for 1 h and allowed to equilibrate at 25 °C for 4 h. Subsequent to these preparations, the sample homogenate underwent a derivatization process. Post-derivatization, the samples underwent centrifugation at 20000 g for 10 min. The supernatant was carefully collected. The collected supernatant was subjected to vacuum drying. To reconstitute the dried samples for subsequent LC-MS (AB SCIEX 4000) analysis, 200 μL of acetonitrile was added.

Comet assay

The assessment of DNA damage in CRC cells was performed using the comet assay, employing a Comet Assay kit (4250-050-K, TREVIGEN, United States). Cells were trypsinized and resuspended in ice-cold phosphate-buffered saline at 2×10^5 cells/mL, and a 50 μL cell suspension was combined with 500 μL preheated comet LMA garose. This mixture was deposited at the center of object slides and allowed to settle for 30 minutes at 4 °C until a distinct 0.5 mm clear ring emerged at the CometSlide™ area edge. Subsequently, slides were immersed in a 4 °C Lysis Solution overnight to enhance sensitivity. After a 30-min wash with neutral electrophoresis buffer (100 mmol/L tris base, 300 mmol/L sodium acetate, pH 9.0), samples underwent electrophoresis at 21 volts for 45 min at 4 °C. Neutral electrophoresis buffer was drained, and slides were submerged in DNA Precipitation Solution for 30 min, followed by a 30-min immersion in 70% ethanol at 25 °C. The dried slides were stained with SYBR green I (S7563, Invitrogen, United States), and images were captured using a Zeiss microscope (LSM 700, Carl Zeiss, Germany).

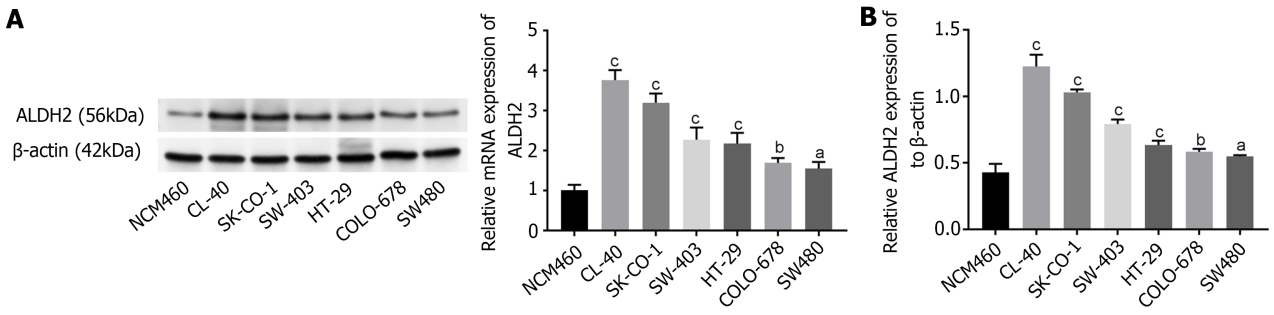

Figure 1 Identifying high expression of aldehyde dehydrogenase 2 family member in colorectal cancer cells. A: Western blot analysis of aldehyde dehydrogenase 2 family member (ALDH2) protein expression in colorectal cancer (CRC) cell lines (CL-40, SK-CO-1, SW-403, HT-29, COLO-678, and SW480) and human normal colon epithelial cell line (NCM460); B: Quantitative reverse transcriptase PCR analysis of ALDH2 expression in human CRC cell lines as indicated. Data are displayed as the mean ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. ALDH2: Aldehyde dehydrogenase 2 family member.

Mice tumorigenesis assay

Mice (5-week-old of age; nude mice, BALB/c; males) were bought from Vital River Laboratories (Beijing, China). We raised mice routinely for one week to adapt to the environment. SK-CO-1 cells (3×10^6) were injected into the mice's inguinal skin. The tumor growth was monitored for 7 d. All mice were randomized into two groups ($n = 5$ per group) and subjected to CVT-10216 treatment (experimental group) or Vehicle (control group) at 50 mg/kg daily after three weeks. After a period of 2 wk, we killed nude mice with an overdose of pentobarbital. All animal experiments were approved by the Animal Ethics Committee of Beijing Viewsolid Biotechnology Co. LTD (VS2126A00173). The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Statistical analysis

GraphPad Prism 7.0 was utilized for analyzing data with an expression of ± SD. We performed single-group comparisons using a student's *t*-test. We analyzed multiple group differences by means of an ANOVA test. $P < 0.05$ showed statistical significance.

RESULTS

Identifying high expression of IGF2 in CRC cells

We analyzed expression levels of ALDH2 in NCM460, CL-40, SK-CO-1, SW-403, HT-29, COLO-678 and SW480 *via* western blot analysis. ALDH2 expression levels were up-regulated in CRC cell lines, including CL-40, SK-CO-1, SW-403, HT-29, COLO-678 and SW480, comparing to that in NCM460 ($P < 0.05$, $P < 0.01$, $P < 0.001$). Only two cell lines (CL-40, SK-CO-1) expressed relatively high levels of ALDH2 (Figure 1A). Moreover, the ALDH2 mRNA expression in CRC cell lines was higher than that in NCM460 ($P < 0.001$, $P < 0.01$, $P < 0.05$; Figure 1B).

ALDH2-deficiency causes ACE accumulation and DNA damage in CRC cells

We transfected CL-40 and SK-CO-1 cells with a shRNA to knock down ALDH2 (sh-ALDH2). We examined the Alda-1's effect (a selective agonist of ALDH2)[15]. Western blotting was utilized to detect cell transfection efficiency (Figure 2A). ALDH2 was lowly expressed in sh-IGF2-transfected cells ($P < 0.001$). while the sh-ALDH2 cells treated with Alda-1 (1 μM), could reverse the down-expressed ALDH2 when comparing with the sh-ALDH2 group ($P < 0.01$). qRT-PCR results revealed the same trend as Western blotting ($P < 0.01$, $P < 0.001$; Figure 2B). We measured the ACE amount in sh-ALDH2 CL-40/SK-CO-1 cells. Sh-ALDH2 indeed caused an increased ACE in CL-40 and SK-CO-1-shALDH2 cells when compared to that in sh-NC cells ($P < 0.001$); Alda-1 treatment could exhibit significantly reduced ACE level as compared to the shALDH2 group ($P < 0.01$; Figure 2C). We examined the γH2AX expression in the transfected cells with or without treatment of Alda-1. sh-ALDH2 exhibited increased levels of γH2AX in CL-40 and SK-CO-1 cells Without treatment ($P < 0.001$). However, under the Alda-1 treatment, sh-ALDH2+Alda-1 cells exhibited reduced γH2AX as compared to sh-ALDH2 group ($P < 0.01$, $P < 0.001$; Figure 2D). We investigated DNA damages in CL-40 and SK-CO-1 cells *via* comet assay. The induced intensive DNA damage in sh-ALDH2 cells was shown, addition of treatment inhibits the DNA damage in CL-40 and SK-CO-1 cells (Figure 2E). ALDH2 could remission exogenous ACE and DNA damage in CRC cells.

ALDH2 silencing promotes apoptosis of CRC cells

We did flow cytometric analysis and western blotting to evaluate the impact of ALDH2 deficiency on CL-40 and SK-CO-1 cell apoptosis. The cells transfected with sh-ALDH2 showed more apoptotic cells, indicating that the decreased expression of ALDH2 led to an increase in CL-40 and SK-CO-1 apoptosis, and Alda-1 treatment could reverse this trend ($P < 0.001$; Figure 3A). Likewise, we detected Bax and Bcl-2 expression levels by western blot assay. sh-AlDH2-transfected cells exhibited lower Bcl-2 expression level and higher Bax expression as comparing to the sh-NC group ($P < 0.001$). Alda-1 treatment reversed the outcome of sh-ALDH2 on Bax/Bcl-2 expression ($P < 0.001$; Figure 3B). Moreover, CCK-8 results

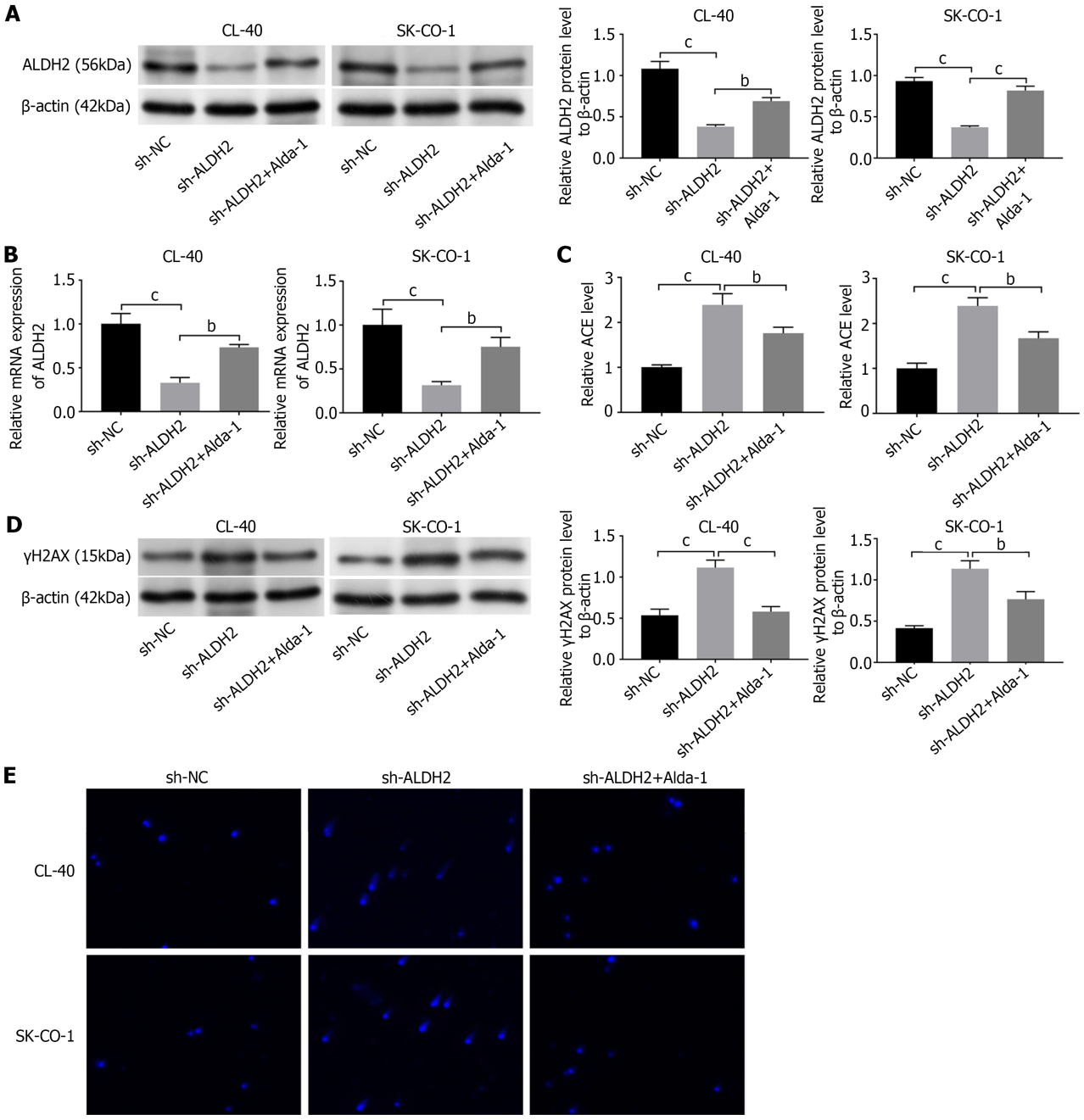

Figure 2 Aldehyde dehydrogenase 2 family member promotes the accumulated acetaldehyde and DNA damage of colorectal cancer cells.
A: Western blot measured the transfection efficiency of shRNA aldehyde dehydrogenase 2 family member (sh-ALDH2) and treated with Alda-1 (1 μM) in CL-40 and SK-CO-1 cells; B: Quantitative reverse transcriptase PCR measured the transfection efficiency of sh-ALDH2 and treated with Alda-1 (1 μM) in CL-40 and SK-CO-1 cells; C: Acetaldehyde quantification of sh-ALDH2 and sh-ALDH2+Alda-1 cells; D: Western blot measured the γH2AX (a DNA-damage response protein) expression of CL-40 and SK-CO-1 cells; E: Comet assay of sh-ALDH2 transfected CL-40 and SK-CO-1 cells that were treated with or without Alda-1. Data are displayed as the mean ± SD. $^{b}P < 0.01$, $^{c}P < 0.001$. ALDH2: Aldehyde dehydrogenase 2 family member.

showed that sh-ALDH2 transfection could inhibit the cell viability in CL-40 and SK-CO-1 cells, while cell viability was evidently increased after treatment of Alda-1 ($P < 0.01$, $P < 0.001$; Figure 3C).

ALDH2 represses JNK/P38 MAPK activation for the inhibition of cell apoptosis of CRC cells

MAPK signal pathway was examined. SP600125 (20 μM), a highly efficient inhibitor of JNK, was added for the inhibition of p-JNK expression in sh-ALDH2 CL-40 and SK-CO-1 cells. SB203580 is a highly selective inhibitor of p38 MAPK[16]; SB203580 (5 μM) was added for the inhibition of the p-P38 MAPK expression in sh-ALDH2 CL-40 and SK-CO-1 cells. Western blot assay demonstrated p-P38 MAPK/P38 MAPK and p-JNK/JNK activation in sh-ALDH2 cell lines ($P < 0.001$), and Alda-1 treatment could reverse this trend ($P < 0.01$, $P < 0.001$). SP600125 treatment could inhibit p-JNK activation by sh-ALDH2 ($P < 0.001$) but did not affect the p-P38 MAPK. SB203580 treatment inhibited sh-ALDH2 activation of p-P38 MAPK ($P < 0.01$) and p-JNK expression (Figure 4A). Flow cytometry showed that sh-ALDH2 promoted apoptosis of CL-40 and SK-CO-1 cells, while Alda-1 treatment, JNK inhibitor (SP600125), and p38 MAPK inhibitor (SB203580) reversed

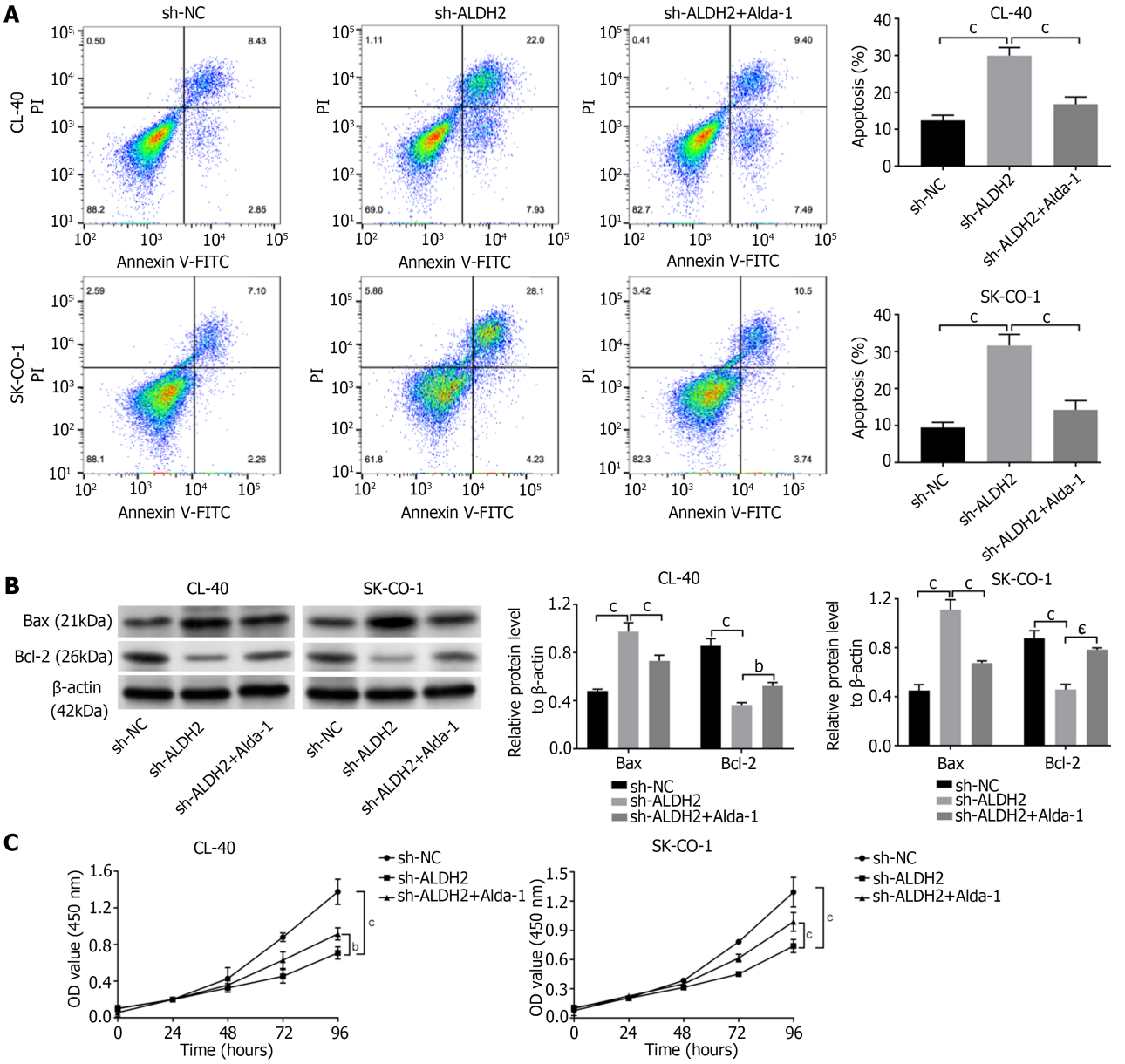

Figure 3 Aldehyde dehydrogenase 2 family member silencing promotes the apoptosis of colorectal cancer cells. A: Flow cytometric analysis of apoptosis of CL-40 and SK-CO-1 cells; B: Detection of apoptosis marker protein (Bax and Bcl-2) in shRNA aldehyde dehydrogenase 2 family member-cells co-treated with Alda-1; C: The cell viability in CL-40 and SK-CO-1 cells was measured by cell counting kit-8. Data are displayed as the mean ± SD. $^{b}P < 0.01$, $^{c}P < 0.001$. ALDH2: Aldehyde dehydrogenase 2 family member.

this phenomenon ($P < 0.001$; Figure 4B). Then, expressions of Bax and Bcl-2 were identified to explore how the MAPK signal pathway influenced cell apoptosis. We found that sh-ALDH2 downregulated Bcl-2 expression and upregulated Bax expression ($P < 0.001$). Moreover, in sh-ALDH2 cells, Bcl-2 expression was obviously activated, while Bax level was decreased with Alda-1, SP600125, or SB203580 treatment ($P < 0.05$, $P < 0.01$, $P < 0.001$; Figure 4C).

ALDH2 repressed MAPK-apoptosis and DNA damage by regulating ACE in CRC cells

We compared the activities of CRC cells in the presence of ACE. After ACE (200 μM) treatment, CL-40 and SK-CO-1 cells with Alda-1 had reduced p-P38 MAPK/P38 MAPK and p-JNK/JNK as compared to that of control cells ($P < 0.001$; Figure 5A). Importantly, cells in ACE presence, flow cytometric analysis showed that Alda-1, JNK inhibitor (SP600125), and p38 MAPK inhibitor (SB203580) treatment could inhibit the apoptosis of CL-40 and SK-CO-1 cells as compared to control cells ($P < 0.001$; Figure 5B). Similar results were obtained by DNA-damage response in γH2AX expression ($P < 0.001$; Figure 5C). CCK-8 assay also demonstrated that when exogenous ACE was added to CL-40 and SK-CO-1 cells, cells with Alda-1, SP600125, and SB203580 had increased proliferation as compared to that of the control group ($P < 0.001$; Figure 5D).

ALDH2-deficiency causes ACE accumulation and increased DNA damage in vivo

We constructed a xenograft model by inoculating SK-CO-1 cells into nude mice. 50 mg/kg CVT-10216 (a selective

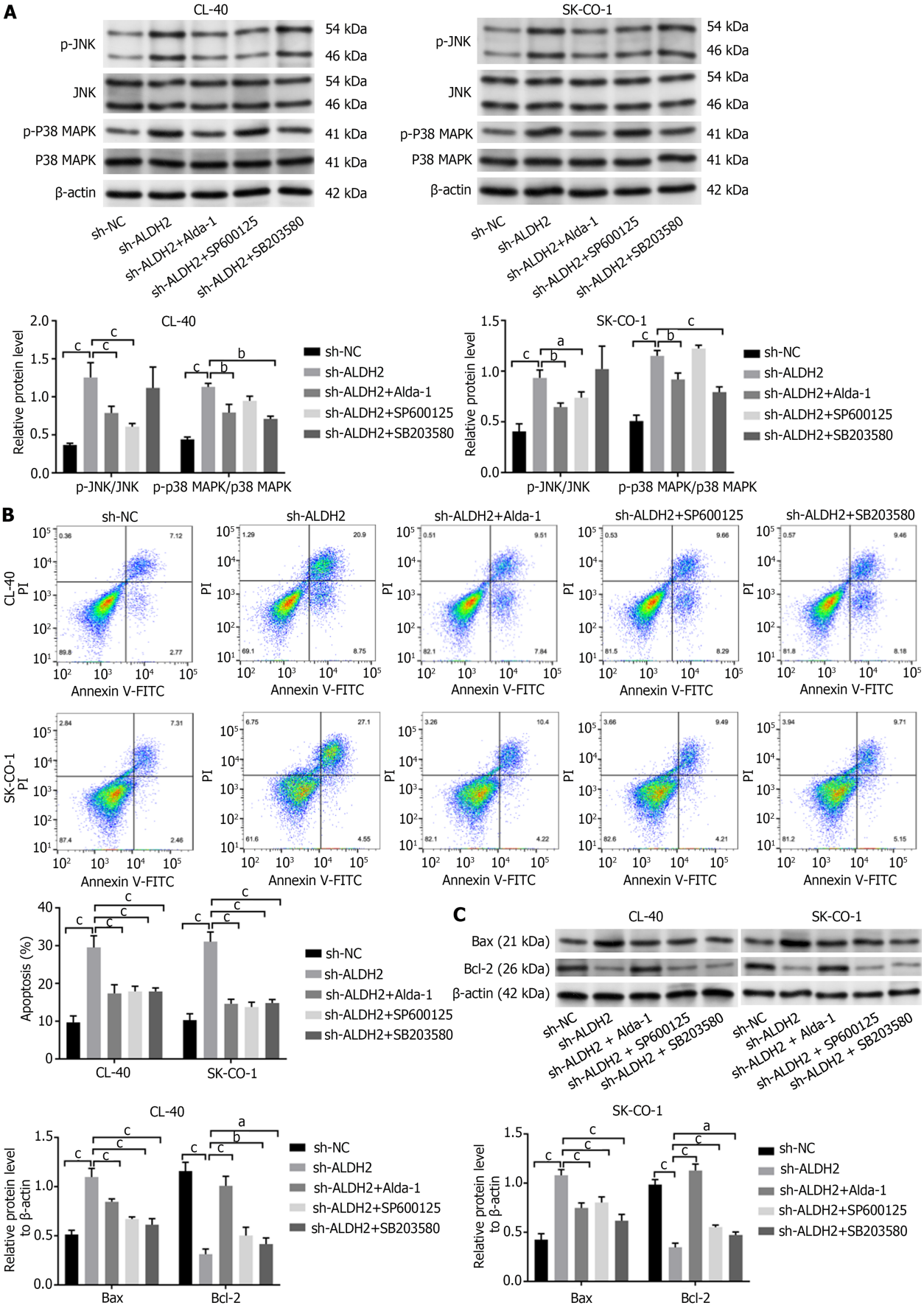

Figure 4 represses MAPK activation to inhibit cell apoptosis of colorectal cancer cells. A: Phosphorylation of JNK and P38 MAPK in CL-40 and SK-CO-1 cells was measured by western blot; B: Flow cytometric analysis of apoptosis of CL-40 and SK-CO-1 cells; C: Detection of apoptosis marker protein (Bax and Bcl-2) in shRNA aldehyde dehydrogenase 2 family member-cells co-treated with Alda-1, SP600125, or SB203580. Data are displayed as the mean ± SD. $^{a}P < 0.05$,

$^{b}P < 0.01$, $^{c}P < 0.001$. ALDH2: Aldehyde dehydrogenase 2 family member.

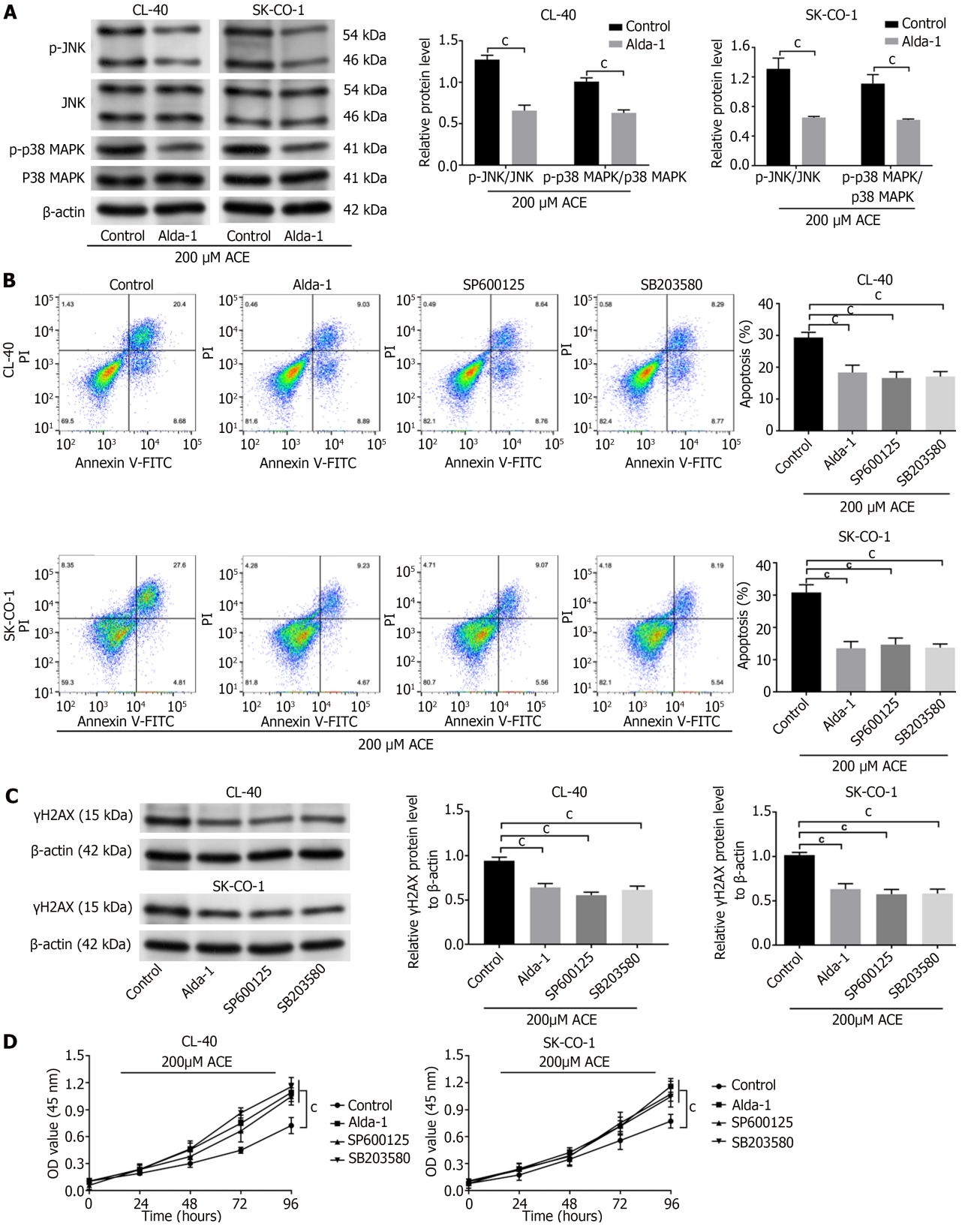

Figure 5 Aldehyde dehydrogenase 2 family member inhibited MAPK-apoptosis and DNA damage by regulating acetaldehyde in colorectal cancer cells. A: Phosphorylation of JNK and P38 MAPK in CL-40 and SK-CO-1 cells in the presence of acetaldehyde (ACE) (200 μM) were measured by western blot; B: Flow cytometric analysis of apoptosis of CL-40 and SK-CO-1 cells in the presence of ACE; C: Detection of protein level of γH2AX in CL-40 and SK-CO-1 cells

with the presence of ACE *via* western blot; D: The cell viability in CL-40 and SK-CO-1 cells with the presence of ACE was measured by cell counting kit-8. Data are displayed as the mean ± SD. $^{c}P < 0.001$.

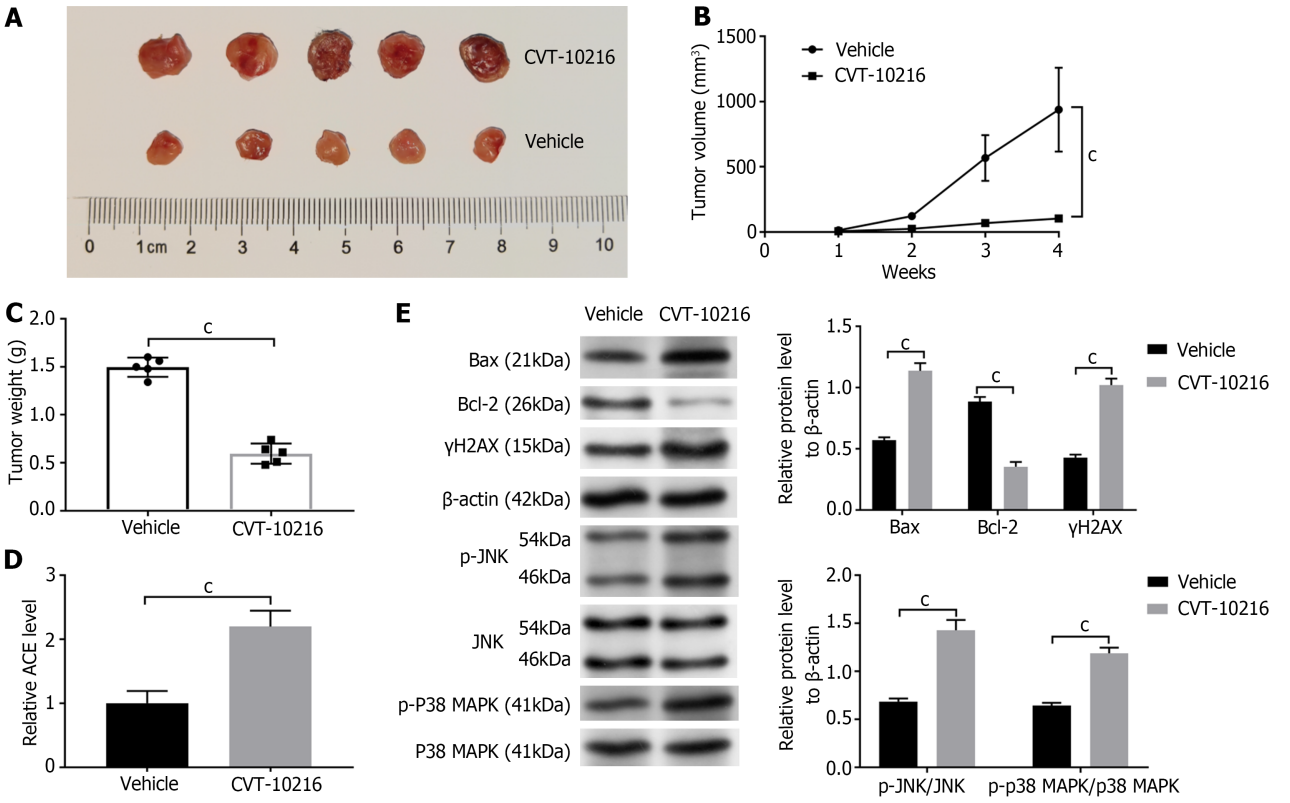

Figure 6 Aldehyde dehydrogenase 2 family member-deficiency leads to accumulated acetaldehyde and increased DNA damage *in vivo*. A: The tumor growth in xenograft tumor mice model; B: The tumor volumes in shRNA aldehyde dehydrogenase 2 family member-SK-CO-1 mouse xenograft models treatment of imatinib; C: The mice were killed, and the tumor weight was assessed; D: Relative quantification of acetaldehyde of mice tumor tissues; E: Detection of Bax, Bcl-2, γH2AX, p-JNK/JNK, and p-P38 MAPK/P38 MAPK protein levels in tumor tissue by western blot. Results are the mean ± SD of triplicate samples. *t*-test, $^{c}P < 0.001$.

ALDH2 inhibitor) was used in mice once a day in the CVT-10216 group, and the control mice were treated with Vehicle once a day. Administration lasted for about two weeks. In the SK-CO-1 mouse xenograft models, tumor growth and volume were inhibited by CVT-10216 as compared to that of the Vehicle group ($P < 0.001$; Figure 6A-C). We measured ACE levels in the tumor tissues from CVT-10216 and Vehicle mice. The ACE level was significantly higher in the CVT-10216 mouse than in the Vehicle group ($P < 0.001$; Figure 6D). In addition, the western blot has submitted that after treatment with CVT-10216, the tumor of mice had decreased Bax/Bcl-2, p-P38 MAPK/P38 MAPK, γH2AX, p-JNK/JNK, and levels than that in Vehicle group ($P < 0.001$; Figure 6E).

DISCUSSION

CRC is an important cause of cancer-related deaths. CRC occurrence is closely associated with genetic factors, ulcerative colitis, intake of tobacco and alcohol, viral infections, environmental factors, *etc*[17]. Our study reported that inhibition of ALDH2 expression caused ACE accumulation and DNA damage in CRC cells and demonstrated that ALDH2 enhanced metastasis in CRC *via* suppression of accumulated ACE and DNA damage by activating the JNK/p38 MAPK pathways.

ALDH2 is expressed highly in patient tumor tissues consuming extreme alcohol[18]. ALDH2 is responsible for ACE metabolism to acetate[19]. ALDH2 reduction increased cell proliferation and stemness and enhanced DNA damage and migration through ACE accumulation in lung adenocarcinoma[11]. In addition, we observed highly expressed ALDH2 in tumor tissues from CRC patients with alcohol drinking history than non-drinkers[17]. The malignant features of CRC cells, including proliferation, apoptosis, ACE level, and DNA damage, were caused by ALDH2 silencing, which can then be reversed by the Alda-1. Alda-1 is a selective agonist of ALDH2[15].

ALDH2 plays a significant role in attenuating cell apoptosis. ALDH2 overexpression regulated autophagy, mitigating apoptosis of renal tubular epithelial cells and renal injury[20]. ALDH2 could decrease 4-HNE, inhibit the MAPK signaling pathway, and decreased apoptosis on liver injury[21]. Research has found that JNK and P38 MAPK pathways activation can induce cell apoptosis in hepatocellular carcinoma[22]. Moreover, ALDH2 represses the JNK/p38 MAPK activation to

inhibit cell migration and proliferation in lung adenocarcinoma[13]. In our study, phosphorylated JNK and p38-MAPK expressions and cell apoptosis were observed by ALDH2 silencing. And ALDH2 repression induced apoptosis in both CL-40 and SK-CO-1 cells by decreasing the expression level of Bcl-2 and increasing the expression levels of Bax. On the other hand, JNK inhibitor SP600125 and p38-MAPK inhibitor SB203580 attenuated ALDH2-induced apoptosis. Likewise, P38/JNK MAPK signaling has participated in licochalcone B[23] and Echinatin[24] induced apoptosis in CRC cells by increased the protein level of Bax, and decreased the expression of Bcl-2. Thus, we concluded that ALDH2 repression could promote apoptosis through activating JNK/P38 MAPK pathways in CRC cells.

ACE can relate to DNA to form diverse types of adducts, which leads to carcinogenesis-related genetic mutations[25]. IARC has designated ACE to be a group I human carcinogen in 2009[26]. ACE is metabolized to acetate by ALDH2, and ALDH2's ability to repress cellular ACE levels is consistent in colon and pancreatic cancers. In heavy ethanol drinkers, it is supported by the connotation of ALDH2 Lack with a high occurrence of CRC and pancreatic cancer[6,27]. To verify whether ACE is involved in ALDH2 regulation in CRC cells by ALDH2, we treated cells with ACE and indicated that ALDH2 could inhibit the JNK/P38 MAPK-apoptosis and DNA damage by regulating ACE, thereby affecting the cell viability in CRC. Moreover, CVT-10216 is a highly selective, reversible inhibitor of ALDH-2 that reduces excessive alcohol drinking[28]. CVT-10216 significantly decrease migration and stemness properties of CRC cells[29]. In our *in vivo* study, CVT-10216 treatment also caused accumulated ACE, high DNA damage, and tumor growth in mice. Thereby, both *in vivo* and *in vitro* experiments have confirmed that ALDH2 repression caused the accumulation of ACE, and induced cell apoptosis and DNA damage in CRC.

CONCLUSION

In conclusion, our work demonstrated that ALDH2 repression caused the accumulation of ACE, inducing cell apoptosis and DNA damage by means of activating the JNK/p38 MAPK signaling pathway in CRC. ALDH2 is utilized as a therapeutic target for reversing patients with CRC.

FOOTNOTES

Author contributions: Yu M, Lu YP designed the study; Chen Q and Lu YP collected and analyzed the data; Yu M wrote the manuscript. All authors reviewed and approved the final manuscript.

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