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Article

Acetate Decreases PVR/CD155 Expression via PI3K/AKT Pathway in Cancer Cells

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Keywords: *PVR/CD155; Colorectal Cancer; Short-chain fatty acid, Acetate, CD8*$^{+}$

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ABSTRACT

In recent years, a growing interest has been garnered in the restoration of anti-tumor immunity in cancer treatment. As a potential therapeutic strategy, the benefits of immune checkpoint inhibitors have been demonstrated in many clinical studies. Although various methods have been applied to suppress immune checkpoints to boost anti-tumor immunity, including immune checkpoint inhibitors, there still exist unmet clinical needs to improve the response rate of cancer treatment. Here, we show that acetate suppresses the expression of a ligand for immune checkpoint, poliovirus receptor (PVR/CD155), in colon cancer cells. We demonstrated that acetate treatment in the cancer cells enhances effector responses of $CD8^+$ T cells via reduced expression of PVR/CD155. We also found that acetate reduces the expression of PVR/CD155 through the PI3K/AKT pathway deactivation. Together, we demonstrated that acetate mediated the expression of PVR/CD155 in cancer cells that might potentiate the anti-tumor immunity in the cancer microenvironment. Our findings indicate that maintaining particular acetate concentrations could represent a complementary treatment strategy in current cancer treatment.

1. INTRODUCTION

Colorectal cancer is one of the most highly ranked cancers causing death in the U. S. (1). Booming the anti-PD-1 blockade antibody treatment to various cancers, the pembrolizumab, an anti-PD-1 antibody, has applied to a small proportion of colorectal cancer patients with microsatellite instability-high tumors (2). Although the approach of anti-PD-1 antibody therapy improved median overall survival in mismatch-repair deficiency colorectal cancer patients, mismatch-repair proficient patients were not reached (3). Many other immune checkpoint inhibitors have been developing to broaden the clinical benefits of immunotherapy, however, new immune-modulating strategies offering enhanced therapeutic outcomes are still needed.

The microbial metabolites, short-chain fatty acids (SCFA), have shown to be involved in normal gastrointestinal functions, including immune regulation and host metabolism. SCFA are fatty acids containing fewer than six carbons produced during the digestion of indigestible fiber by gut microbiota (4). As the most abundant SCFAs, acetate represents about 50% of SCFAs in the colon and maintaining acetate concentration is crucial for normal colon homeostasis (4). The concentration of acetate in feces from a healthy adult is 30~50 mM (5), while altered acetate concentration has been shown in various diseases. The high concentration of acetate has shown to induce apoptosis in colorectal cancers (6). Also, the reduced concentration of acetate was found in the fecal of colorectal cancer patients (7). Although the negative correlation between colorectal cancers and acetate concentration has been found, the detailed molecular mechanisms associated with an immunological function of acetate is unclear.

As a member of the nectin-like molecule family, CD155 functions as a receptor of poliovirus. A poliovirus receptor (PVR)/ CD155 is a Type1 transmembrane glycoprotein in the immunoglobulin superfamily. It has been shown that PVR/CD155 is frequently overexpressed in human malignant cancers. Overexpressed PVR/CD155 promotes cancer cell survival and migration (8). The function of PVR/CD155 also has been studied in cancer immune evasion. As the ligand for T-cell immunoreceptor with Ig and ITIM domains (TIGIT), PVR/CD155 expressed on either DC or cancer cells binds to TIGIT and suppresses T cell activation. Due to its immune escape function, PVR/CD155 recently has gained great interest as a therapeutic target for cancer immunotherapy.

Here, we identify acetate as a potent anti-cancer immunomodulatory molecule that can suppress the expression of PVR/CD155, a ligand for TIGIT, in colorectal cancer cells. We demonstrated that acetate treatment in the cancer cells enhances effector responses of $CD8^+$ T cells via reduced expression of PVR/CD155. Our study reveals that acetate reduces the PVR/CD155 expression via PI3K/AKT deactivation. Together, we demonstrated that acetate regulates the expression of PVR/CD155 through PI3K/AKT pathway in cancer cells, thereby enhancing the effector responses of $CD8^+$ T cells. These results suggest that targeting strategies considering acetate concentration could provide benefits to the treatment of solid tumors, including colon cancers.

2. RESULTS

2.1. Inhibition of colorectal cancer cell proliferation by short-chain fatty acids

Short-chain fatty acids are known to be cytotoxic in cancers, while the function of short-chain fatty acids in anti-cancer immunity is poorly understood (6). Physiological concentrations of short-chain fatty acids are found in the human large intestine ranging from 43.5 to 63.4mmol/kg for acetate, from 14.2 to 26.7mmol/kg for propionate, and from 14.7 to 24.4mmol/kg for butyrate (4). Among acetate, propionate, and butyrate, the highest concentrations were found for acetate, followed by propionate and butyrate in the colon (9). To explore the function of short-chain fatty acids in cancer progression, we first checked the cell viability with the treatment of each short-chain fatty acid at varying concentrations representing a low, medium, and high physiological level of short-chain fatty acids ranging from 10mM to 100mM. Consistent with previous research, the viability of colorectal cancer cells, HCT116, HT-29, and SW-480, was decreased upon SCFA treatment in a dose-dependent manner (Figure 1A). Even though the physiological concentration of acetate may inhibit colorectal cancers evidenced by the data shown in Figure 1A, the function of acetate as a therapeutic target needs to be further investigated. Among the cancer cells, the viability of SW-480 was most decreased upon acetate or propionate treatment. HT-29 showed the highest reduction of viability upon butyrate treatment. Additionally, apoptosis was enhanced upon short-chain fatty acids treatment in HCT116 cells in a dose-dependent manner. (Figure 1B). Among SCFAs, acetate was the least cytotoxic at the low concentration, 25mM, showing 85% of the cell viability in HCT116 cells. Taken together, acetate, propionate, and butyrate inhibit cell proliferation and induce apoptosis at the physiological concentrations in colorectal cancer cells.

2.2. Effect of SCFAs on the expression of ligands for immune checkpoints in colorectal cancer cells

To validate the function of short-chain fatty acids in anti-cancer immunity, we first tested the effect of short-chain fatty acids on ligands expression for immune checkpoints. As shown in Fig. 2, each short-chain fatty acid, showed the different regulation on the expression of immune checkpoint ligands. Acetate reduced PD-L1 and PD-L2 low concentration while butyrate and propionate induced PD-L1 and PD-L2 expression (Fig. 2A). Butyrate and propionate slightly reduced mRNA expression of Tim-3 ligands, Galectin-9, and CEACAM1, but acetate increased the expression of Galectin-9 and CEACAM1 at the highest concentration, 100uM (Fig. 2B). A similar pattern was shown in the TIGIT ligand, Nectin-2/CD112, mRNA expression in which the highest concentration of acetate enhanced Nectin-2/CD112 expression. However, mRNA expression of another TIGIT ligand, PVR/CD155, was significantly reduced by all three SCFAs. Acetate reduced 50% mRNA expression of PVR/CD155, and butyrate and propionate decrease about 75% mRNA expression of PVR/CD155 compared to the control (Fig. 2C). Inhibiting interaction between the immune checkpoints and their ligands is the primary strategy in the current anti-cancer immunotherapy. Since we observed the reduced cancer cell viability and the decreased mRNA expression of PVR/CD155 in cancer cells upon all three SCFA (Fig. 1 and 2C), we further confirmed whether PVR/CD155 is crucial in cancer cell survival. As shown in Fig. 2D, the viability was not affected by PVR/CD155 K/D in the cancer cells. As the most frequently mutated oncogene in cancers, KRAS mutation is a biomarker of resistance to anti-EGFR treatment in colorectal cancer. Since HCT116 carries a KRAS mutation, effect of acetate on the PVR/CD155 expression was also tested in other colorectal cancer cell lines with distinct

mutation profiles, HT-29 and CaCo-2 (KRAS WT). As shown, PVR/CD155 mRNA expression were reduced upon acetate treatment in HT-29 and Caco-2 cells (Fig. 2E and F respectively). Consistent with mRNA expression data, the PVR/CD155 protein expression was also decreased with acetate treatment in HT-29 and CaCo-2 (Fig. 2G and H respectively). The results indicate that the regulation of PVR/CD155 by acetate is KRAS mutation independent. Altogether, each SCFA showed different transcript regulation in the ligands for PD-1, Tim-3, and TIGIT. Among them, only PVR/CD155 was reduced by all three SCFA, indicating PVR/CD155 as the most promising therapeutic target by microbiota metabolite.

2.3. Enhanced effector responses of $CD8^+$ T cells via reduced expression of PVR/CD155 in the cancer cells treated with acetate

Among SCFAs, acetate is the most abundant metabolic product of gut microbiota and has been proven as immune modulators in various biological processes such as gut homeostasis (10, 11). Although the function of acetate related to the metabolic regulation in the tumor microenvironment was known, the contribution of acetate in the anti-cancer immunity is not clear. Our data indicate that acetate only inhibits PVR/CD155 expression, while butyrate and propionate decreased the expression of the multiple ligands. Therefore, we decided to focus on the study for the regulation of PVR/CD155 by acetate to understand the more specific molecular mechanism underlying decreased expression of immune checkpoint ligands in cancers. DNAM-1, TIGIT and CD96 are well characterized receptors for CD155 and CD112 (12, 13). Even though the receptor recognition of the ligands and its triggered signal transduction were intensively investigated, the molecular mechanism related with the ligand expression in the cells is not well understood. To verify the role of PVR/CD155 in anti-tumor immunity, we first

inhibited PVR/ CD155 signaling by using blocking antibodies and tested the induction of cancer cell responses by $CD8^+$ T cells. As shown in Figure 3 A, B and C, blocking PVR/CD155 signaling significantly enhances the apoptotic population in HCT116 cancer cells when it co-cultured with $CD8^+$ T cells while the blocking antibody does not affect the cell viability. In contrast, the effect of blocking Nectin-2/CD112 signaling on the apoptosis was marginal in HCT116 cancer cells (Fig. 3B and C). To test the effect of acetate in the regulation of PVR/CD155 expression, we next tested the PVR/CD155 protein expression in cancer cells upon acetate treatment by flow cytometer analysis. Consistent with the reduced mRNA expression by acetate, the protein levels of the PVR/CD155 were decreased, as evidenced by an increased population of $CD155^{low/-}$ with 25 and 50mM acetate treatment (Fig. 3D). We also tested the effect of acetate in the PD-L1 protein expression to see any compensatory effect against the decreased PVR/CD155 expression. However, the protein expression of PD-L1 was low, and the expression changes after the acetate treatment were negligible in our model system, HCT116 cells (Figure 3D). Tumor infiltrating T cells are mostly effector or memory cells. To investigate the effect of acetate in intrinsic regulation of memory $CD8^+$T cell responses, $CD8^+$ T cells were co-cultured with acetate treated HCT116 at a ratio of 1:1. Acetate treatment on HCT116 increases IFN-γ production of $CD8^+$ T cells compared to the T cells stimulated with a CD3/CD28 alone, supporting enhanced effector responses of $CD8^+$ T cells by reduced expression of PVR/CD155 (Fig. 3E). Furthermore, the expression of Granzyme B and Perforin was also enhanced when $CD8^+$ T cells were co-cultured with acetate treated HCT116 (Fig. 3E). Even though the reduced expression of PVR/CD155 by acetate was observed, only about 15% of the total cells showed the responses, indicating that other immunomodulatory molecules may have to be involved in inducing sufficient

cytotoxic response. However, we believe that acetate as the most abundant metabolite in the colon might play a role in anti-cancer immunity as evidenced by the expression regulation of ligands for immune checkpoints in Figure 2A, B and C. Detailed molecular mechanism related to the decreased expression of PVR/ CD155 upon acetate treatment and its compensatory or synergistic crosstalk with other immunomodulatory molecules have to be further investigated. Taken together, these findings suggest that acetate reduces PVR/CD155 expression at both the transcriptional and protein levels in the colorectal cancer cells and enhances IFN-γ production of $CD8^+$ T cells that might contribute to an anti-cancer immunity in the cancer microenvironment.

2.4. Inhibition of PI3K decreases PVR/CD155 expression in colorectal cancer cells

To further investigate the underlying mechanism of how acetate down-regulates PVR/CD155 expression in colorectal cancer cells, we next checked the downstream signaling molecules upon acetate treatment in HCT116 cells. As shown in Figure 4A, acetate decrease pAKT expression. Therefore, we next tested the PVR/CD155 expression in the cancer cells after the treatment of PI3K inhibitor, LY294002 (14). As expected, the PVR/CD155low/- population was increased when the 7AAD- live population was analyzed after 10uM LY294002 treatment in HCT116 cells, indicating that the PI3K/AKT pathway is involved in the regulation of PVR/CD155 expression (Fig. 4B). TLR-mediated activation of the transcription factor NF-kB has been shown to up-regulate PVR/CD155 expression (15). To test whether the reduced expression of PVR/CD155 by acetate was also dependent on inactive NF-kB signaling, we analyzed the cells after anacardic acid, the inhibitor of histone acetyltransferase of P65 (16). However, inactivation of NF-kB pathway was not able to increase the $PVR/CD155^{low/-}$ population in the cancer cells even though acetate down-regulate pp65 expression (Fig. 4A and B). To

test the possibility that the cell death induced by LY294002 may affect the result, we tried to exclude 7AAD+ dead population and only analyzed 7AAD- population to confirm PVR/CD155 low population upon LY294002 treatment. In Figure 4C, LY294002 still increases the PVR/CD155$^{low/-}$ population. These results are consistent with the previous evidence that CD155 expression is regulated by the PI3K/AKT pathway and also support that the acetate can trigger this pathway. (17, 18).

3. DISCUSSION

In recent years, increased attention has been garnered in immune checkpoint inhibitor therapy due to its remarkable clinical responses. However, despite the remarkable clinical responses, only a limited number of cancer patients respond to the current therapeutics (2). Therefore, various therapeutic strategies were proposed to increase the clinical responses of immunotherapy in cancers (19). Blocking TIGIT, an immune checkpoint protein on T cells and natural killer cells has been emerged as a promising strategy to control anti-tumor immunity in cancers (20). TIGIT on T cells has shown to bind to ligands, including Nectin-2/CD112 and PVR/CD155 on cancers, thereby inhibiting the cytotoxic function of T cells in a tumor microenvironment (20). Since cancers escape the anti-tumor immunity by overexpressing the immune checkpoint ligands (21), limiting expression of immune checkpoint ligands on cancer cells could be one of the effective therapeutic strategies, leading the successful clinical outcomes.

In this study, our result shows the potential of acetate as an efficient immune modulator via reducing the expression of the immune checkpoint ligand, PVR/CD155, in cancer cells. Acetate treatment on cancer cells decreases PVR/CD155 expression through the PI3K/AKT deactivation, thereby enhancing effector responses of $CD8^+$ T cells (Fig. 4D). Although we confirmed the downregulation of PVR/ CD155 by acetate in cancer

cells, further investigation is required to elucidate the detailed mechanism associated with the transcriptional and translational regulation of PVR/CD155 in PI3K/AKT pathways. We also revealed that the production of cytotoxic molecules secreted by $CD8^+$ T cells, IFN-γ, were significantly enhanced by acetate treatment in cancer cells, supporting the function of acetate in anti-cancer immunity in the tumor microenvironment. With great efforts to broaden the clinical benefits of cancer patients, modulating tumor microenvironment by acetate can be used to complement current immune therapies with a different mechanism.

4. MATERIALS AND METHODS

4.1. Colorectal cancer lines and human $CD8^+$ T cells

HCT116 (HLA-A2-positive) was cultured in RPMI1640 media containing L-glutamine, 10% fetal bovine serum, 1% penicillin-streptomycin (all from GIBCO) at 37 ℃ 5% CO_2 condition. HCT116 was purchased from ATCC. Human $CD8^+$ T cells (HLA-A2-positive) were purchased from STEMCELL. The cells were expanded by a 1:10 ratio of beads from the human T cell activation/Expansion kit (Miltenyi Biotec). For activation of $CD8^+$ T cells, the T cells were treated with beads from the human T cell activation/Expansion kit at a 1:2 ratio for three days. Colorectal cancer lines were co-cultured with activated $CD8^+$ T cells at a 1:1 ratio for two days(22-24).

4.2. Short-chain fatty acid (SCFA) treatment

Acetate, butyrate, and propionate were diluted by distilled water with sodium acetate (S5636, Sigma Aldrich), sodium butyrate (P5436, Sigma Aldrich) and sodium propionate (B5887, Sigma Aldrich) then filtered by 0.22 μm syringe filter. The treatment of acetate

(25, 50, and 100 mM), butyrate and propionate (10,20 and 40mM) exogenously into HCT116, was performed for one day. The change of immune-suppressive molecules by short-chain fatty acid was analyzed by flow cytometry, and real-time PCR. Relative quantification of target genes was performed by qPCR and the $2^{-\Delta\Delta Ct}$ method. Target gene expression was normalized to the GAPDH mRNA level.

4.3. Flow cytometry measurement

The cell death of HCT116 was measured with AnnexinV-APC (Biolegend) and 7AAD (BD Biosciences). To test the effector response of $CD8^+$ T cells, activated $CD8^+$ T cells were co-cultured with HCT116 cells which is pre-treated with (25 or 50mM, for 24hrs) for 48hrs. Intracellular staining was performed according to the manufacturer`s instructions (BD Biosciences). Briefly, $CD8^+$ T cells were treated with Golgi stop (BD Biosciences), phorbol 12-myristate 13-acetate (Invivogen) and Ionomycin (Sigma-Aldrich) for 6 hr. For flow cytometric analysis of $CD8^+$ T cells, anti-human granzyme B-FITC (GB11, BD Pharmingen), CD8a-PE (SK1), IFNγ-APC (4S.B3) and perforin-PerCP-cy5.5 (B-D48) antibodies (all from Biolegend) were used. Cancer cells were stained with anti-human CD112-PE (TX31) or CD155-APC (SKII.4) antibodies (all from Biolegend). All samples were analyzed by flow cytometry (CytoFlex, Beckman Coulter) and FlowJo software (Tree Star).

4.4. The measurement of the viability of HCT116

The viability change of HCT116 by acetate was measured with CCK8 (DOJINDO). Absorbance was measured at 450 nm using a microplate reader (GloMax® Discover Microplate Reader, Promega).

4.5. Measurement of mRNA level using qPCR

Total RNA of acetate treated cells or siRNA treated samples were extracted using Trizol (Thermo Fisher Scientific). cDNA was synthesized using SuperScript VILO™ Master Mix (Invitrogen). RT-qPCR was performed with TB Green Premix Ex Taq™ (TaKaRa). Primers listed below were synthesized by Bioneer Inc. (Daejeon, Korea).

Genes		Sequence (5'-3')	Size (bp)
hPDL1	Forward	AAATGGAACCTGGCGAAAGC	104
	Reverse	GATGAGCCCCTCAGGCATTT	
hPDL2	Forward	GTCTTGGGAGCCAGGGTGAC	99
	Reverse	TGAAAAGTGCAAATGGCAAGC	
hCD112	Forward	TCCGGCTATGATGACAACTG	193
	Reverse	TGCAGACGAAGGTGGTATTG	
hCD155	Forward	CCAACATGGAGGTGACGCAT	210
	Reverse	GGCAGGTGTAGTTGCCTTCA	
hCEACAM1	Forward	GAGTAGTGGCCCTGGTTGCTC	85
	Reverse	CGCTGGTCGCTTGCCCT	
hGalectin-9	Forward	CTTTCATCACCACCATTCTG	91
	Reverse	ATGTGGAACCTCTAAGCACTG	
hGAPDH	Forward	GAAGGTGAAGGTCGGAGTC	172
	Reverse	GAAGATGGTGATGGGATTT	

4.6. Inhibition Assay

For inhibition of CD112 or CD155 on HCT116, anti-human CD112 (TX31) or CD155 (SKII.4) antibodies were treated to HCT116 for 6 hours. After PBS washing, activated $CD8^+$ T cells were co-cultured for two days. The cell death of HCT116 was measured by flow cytometry. For inhibition of NF-κB signaling, anacardic acid or LY294002 (all from Abcam) 10 μM were treated into HCT116 for two days. The change of CD112 and CD155 was measured by flow cytometry. For inhibition of CD155 using small interfering RNA (siRNA), siRNA targeting gene of interest or control siRNA (Bioneer) were transfected using the Neon™ system (Invitrogen). All the knockdown was carried out for 48 hours and the knockdown efficiency was confirmed by qPCR. siRNAs were purchased from by Bioneer Inc. (Daejeon, Korea).

4.7. Protein analysis

To confirm the protein expression of signaling proteins, the cells were disrupted by RIPA buffer containing protease inhibitor cocktail (Abcam). The amount of total protein in the supernatant was determined by Pierce™ BCA Protein Assay Kit (Thermo Scientific). Samples were boiled at 100 °C for 5 min, separated by gradient gel (Bio-rad) and transferred onto a polyvinylidene difluoride (PVDF) (Bio-rad) followed by blocking with 10% BSA in TBST (TBS with 0.1% Tween 20) at room temperature for 1 hour. Target signaling proteins, for example, anti-pP65(S536 (93H1)), P65(D14E12 8242S), pAkt(T308, 4056S)), Akt(9272S), , pERK(T202/Y204), ERK(135F5, 4695S) (all antibodies from Cell Signaling), Beta-actin (Santa Cruz Biotechnology) antibodies were incubated with various concentration of BSA in TBST at room temperature for 1 hour. The immunoblots were visualized by Amersham™ ECL™ Prime Western blotting detection reagent and then analyzed by LAS-3000 (Fujifilm).

4.8. Statistical Analysis

All experiments were performed independently at least three times. Values are expressed as mean ± SD. Significance was analyzed using a two-tailed, unpaired *T*-test. A *p*-value of less than 0.05 was considered statistically significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

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CONFLICTS OF INTEREST

The authors declare that they have no competing financial interests.

FIGURE LEGENDS

Figure 1. Cell viability upon SCFA stimulation at varying concentrations. Viability of HCT116, HT-29 and SW-480 cancer cells upon acetate (25, 50 and 100mM), butyrate or propionate (10, 20 and 40mM) treatment was measured by CCK8 assay (A) AnnexinV and 7AAD were analyzed in HCT116 after varying concentrations of acetate, butyrate and propionate by flow cytometry (B). The p-value was calculated by a t-test (** $P < 0.01$, *** $P < 0.001$)

Figure 2. Effect of SCFA on ligand expression for immune checkpoints in colorectal cancer cells. Real-time PCR for mRNA of immune checkpoint ligands was performed in HCT116 cancer cells after the treatment of acetate, butyrate, or propionate. (A, B and C) CD155 K/D was confirmed by real-time PCR and the cell viability was measured by CCK-8 assay (D). mRNA expression of CD155 in HT-29 (E) and Caco-2 (F) cancer cells after the acetate treatment. The protein expression of CD155 was measured by flow cytometry in HT-29 (G) and Caco-2 (H) cancer cells after the acetate treatment. Both low and negative populations were gated in CD155 (CD155low/-) based on pre-gated live cells (7AAD-). The p-value was calculated by a t-test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Figure 3. Enhanced effector responses of $CD8^+$ T cells via reduced expression of PVR/CD155 in the cancer cells treated with acetate. (A) Viability of HCT116 after treatment of CD112 or CD155 blocking antibodies was measured by CTG assay. (B)(C) The analysis of AnnexinV and 7AAD was performed in HCT116, which co-cultured with $CD8^+$ T cells after blocking by CD112 (a112) or CD155 (a155) antibodies. (D) The expression of CD155 and PD-L1 were measured in HCT116 cancer cells after the acetate treatment. Both low and negative populations were gated in CD155 ($CD155^{low/-}$) based on pre-gated live cells ($7AAD^-$). (E) The expression of GranzymeB, Perforin, and IFN-γ

was measured in $CD8^+$ cells, which co-cultured with acetate pre-treated HCT116. The p-value was calculated by a t-test (** P < 0.01, *** P < 0.001)

Figure 4. The function of acetate as a repressor of PVR/CD155 expression via the PI3K/AKT signaling pathway. (A) pP65 and pAKTwere detected upon 50mM acetate treatment in HCT116 by WB, 20 ug of cell lysate per sample (B) The expression of CD155 was measured after the tpereatment of LY294002 10 μM or anacardic acid. Both low and negative populations were gated in CD155 (CD155low/-) based on pre-gated live cells (7AAD-). Asterisks indicate significant differences compared with Acetate 0 mM as a control. (C) PVR/CD155 expression upon LY294002 or anacadic acid treatment (Right). The 7AAD+ dead cells were excluded to confirm the CD155 low population after LY294002 treatment by flow cytometry (Left). (D) The scheme describes the predicted function of acetate in anti-tumor immunity. Acetate inhibits PI3K/AKT, thereby reducing PVR/CD155 expression in cancers. The decreasing PVR/CD155 enhances effector responses of $CD8^+$ T cells releasing GranzymeB, Perforin, and IFN-γ. The p-value was calculated by a t-test (** P < 0.01, *** P < 0.001)

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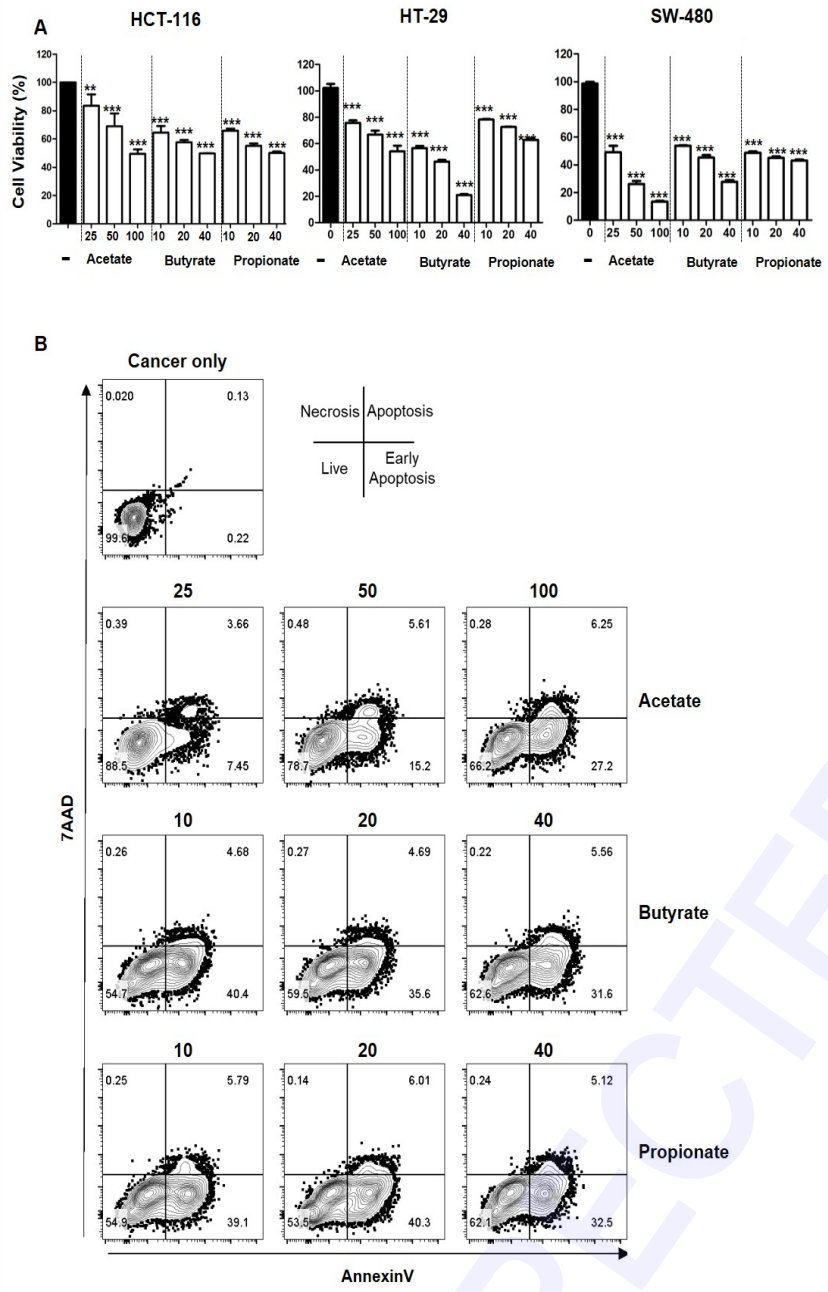

Fig. 1.

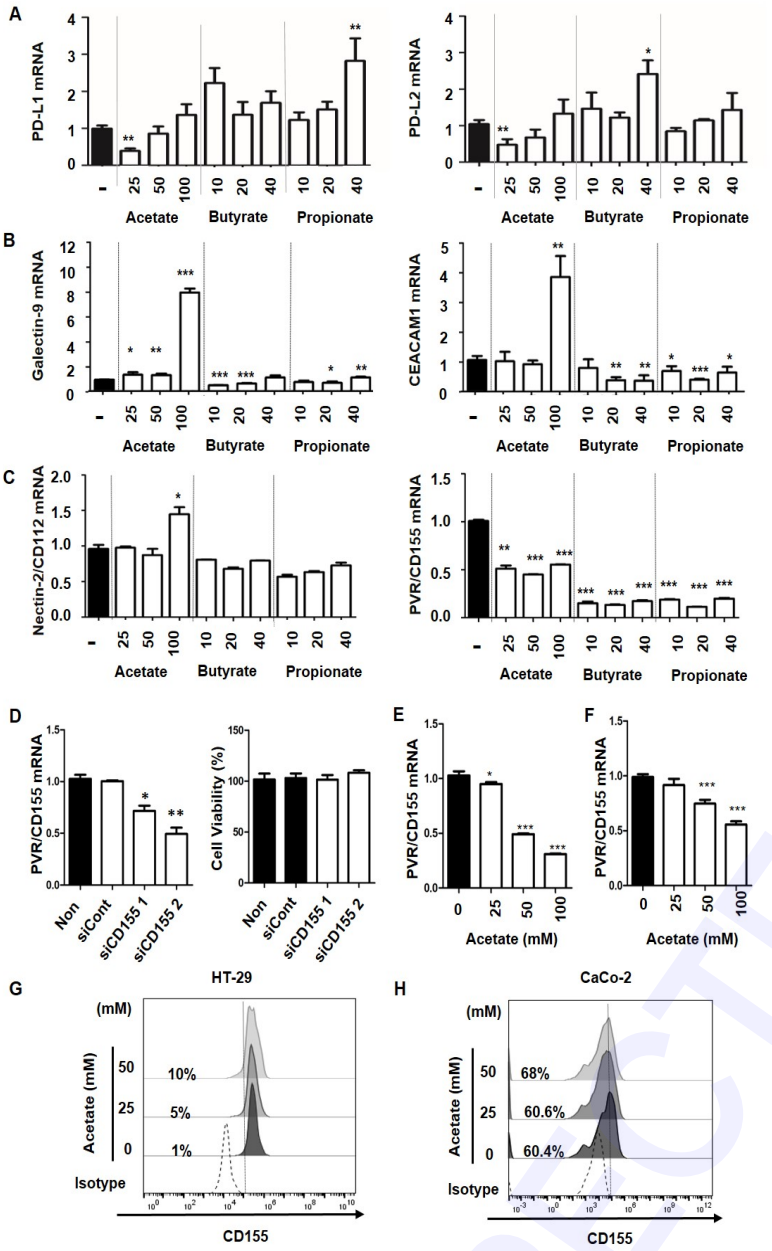

Figure 2.

Fig. 2.

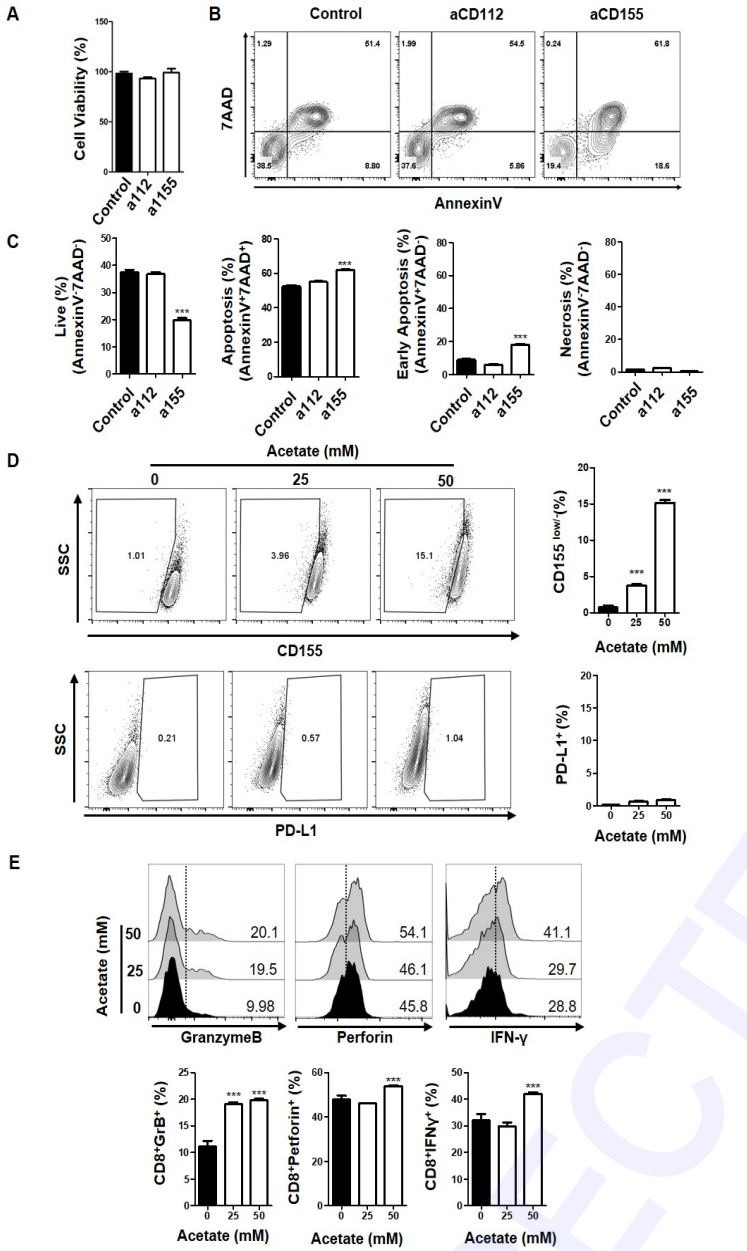

Figure 3.

Fig. 3.

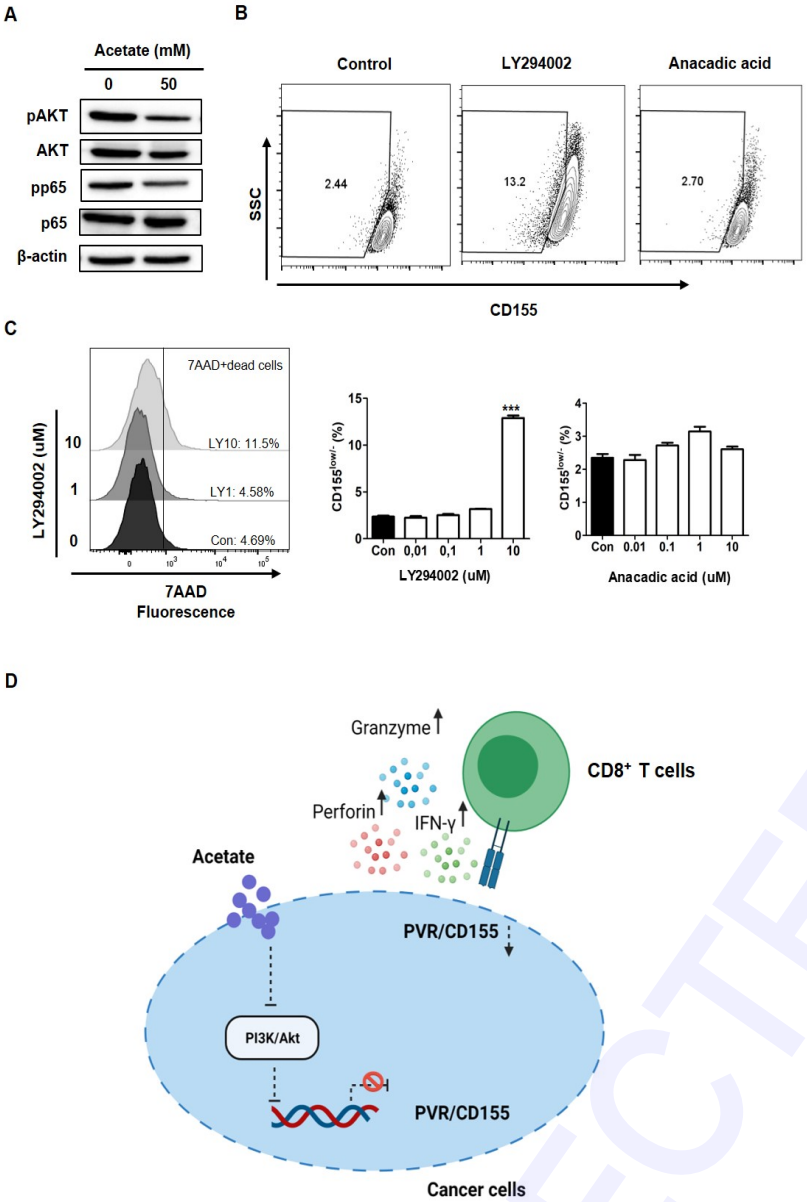

Figure 4.

Fig. 4.