RESEARCH ARTICLE

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Butyrate-mediated Resistance to Trichostatin A Accompanied by Elevated Expression of Glucose Transporter 3 (GLUT3) in Human Colorectal Carcinoma HCT116 Cells

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Abstract

Objective: The aim of study was to investigate the correlation of GLUT3 upregulation and butyrate-mediated acquired chemoresistance. **Method:** A butyrate-resistant CRC cell model was established from parental (PT) HCT116 cells by gradually increasing the concentration of sodium butyrate (NaBu), followed by evaluation of resistance to butyrate and trichostatin A (TSA) by the MTT method. The expression of *SLC2A3* gene and GLUT3 protein were assessed by semi-quantitative RT-PCR and western blotting, respectively. The correlation of GLUT3 and butyrate-induced acquired chemoresistance was investigated using *SLC2A3* silencing. **Results:** Butyrate-resistant (BR) HCT116 cells were more tolerant to butyrate-induced cell death and also resist to 750 and 1000 nM TSA when compared with HCT116-PT cells ($p < 0.05$). Long-term exposure to butyrate revealed that upregulation of the *SLC2A3* gene was significantly increased by more than 20 fold ($p < 0.01$), and that of GLUT3 was elevated by approximately 2 fold ($p < 0.05$) in HCT116-BR cells. Silencing of the *SLC2A3* gene increased the sensitivity of HCT116-BR cells to the effects of TSA. **Conclusion:** Upregulation of GLUT3 is associated with resistance to butyrate and TSA. GLUT3 is a molecular target for the detection of chemoresistant CRC cells and thus a potential target for diagnostic strategies.

Keywords: Butyrate- Chemoresistance- Colorectal cancer- GLUT3- Trichostatin A

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Introduction

Colorectal cancer (CRC) is the most frequently diagnosed cancer and a leading cause of mortality in patients worldwide (Xi and Xu, 2021). Its prevalence often correlates with non-genetic factors, especially lifestyle behaviors and dietary factors that are more potent than genetics (Rawla, Sunkara, and Barsouk, 2019). Although intake of a high-fiber diet has been recommended to reduce the tumor burden in animal models (Donohoe et al., 2014), studies in humans have been inconclusive (Park et al., 2005; Doyle, 2007; Pericleous, Mandair, and Caplin, 2013).

Butyrate is a short-chain fatty acid (SCFA) produced from fermentation of dietary fiber and resistant starch by the gut microbiota. Like other histone deacetylase (HDAC) inhibitors, butyrate exhibits anti-tumor effects by inhibiting cell proliferation (Zeng et al., 2020) and inducing apoptosis (Wang et al., 2016; Cao et al., 2019; Wang et al., 2020) via epigenetic strategies. However, it has also been reported that a butyrate-rich colonic microenvironment contributes to tumor aggressiveness, resulting from the adaptation of a subpopulation of butyrate-challenging CRC cells (Serpa et al., 2010). Moreover, chronic exposure to butyrate leads to resistance to its effects and mediates acquired resistance to chemotherapeutic agents, especially HDAC inhibitors (Kang et al., 2016). Trichostatin A (TSA), an HDAC inhibitor, has been used in the treatment of neurodegenerative and autoimmune diseases (Xu et al., 2011; Regna and Reilly, 2014) and also as a chemotherapeutic agent (Shen et al., 2019; Zhang et al., 2019). However, long-term exposure induced resistance to TSA that involved HIF-1α acetylation (Lee et al., 2018) and Wnt signaling (Wang et al., 2014).

Rapid glucose consumption is a hallmark of cancer. Accumulated evidence revealed that chemoresistant cells often show upregulated expression of glucose transporters (GLUTs) (Cao et al., 2007; Wang et al., 2013; Liu et al., 2014; Jiang, Zhou, Fan, 2018). Overexpression of GLUT1 has been shown to be associated with an unfavorable prognosis in various types of tumors (Wang et al., 2017) and acquired chemoresistance (Liu et al., 2014; Wang et al., 2017), but GLUT3 has not yet been studied extensively. GLUT3, encoded by the *SLC2A3* gene, has the highest affinity among GLUT1, GLUT2, and GLUT4 and is specifically distributed in

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neurons, sperm, white blood cells, and embryos. However, erroneous expression in other tissues is regarded as a sign of oncogenic transformation (Barron et al., 2016). Our previous study found that *SLC2A3* mRNA expression was upregulated 77.2 fold in butyrate-resistant (BR) HCT116 cells and by 2.8 fold in colon cancer stem cells (HCT116-CSCs), when compared with parental HCT116 cells (Khonthun et al., 2020). This evidence reveals that *SLC2A3* is implicated in drug resistance. It has been demonstrated that upregulation of GLUT3 is associated with aggressive behavior in cancer cells and correlated with chemoresistance (Mo et al., 2016; Kuang et al., 2017). As mentioned above, we hypothesized that GLUT3 may be a chemoresistance-related molecule. Therefore, the present study aims to investigate the correlation of GLUT3 upregulation and butyrate-mediated acquired resistance to HDAC inhibitors in a butyrate-insensitive CRC cell model. These findings will reveal chemoresistance-related molecules responsible for drug stress, especially HDAC inhibitors, and will be helpful in predicting chemoresistant cells for early detection and determining guidelines for successful CRC treatment.

Materials and Methods

Cell culture and the establishment of a butyrate-resistant CRC cell model

Human colorectal carcinoma HCT116 cells were obtained from the American Type Culture Collection (ATCC) and grown in Dulbecco's modified Eagle's medium (DMEM) with L-glutamine supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 0.25 µg/ml of amphotericin B at 37ºC in a humidified atmosphere of 95% air and 5% CO_2. A butyrate-resistant (BR) cell model was established by gradually increasing the concentrations of sodium butyrate (0.5–3.0 mM NaBu), which exposed each concentration to every 2 subcultures, and finally, the surviving cells were maintained with media containing 3 mM NaBu for at least 3 months.

Evaluation of the capacity for resistance to butyrate and TSA

Acquired resistance to butyrate and TSA was assessed using an MTT reduction assay. Cells were seeded in 96-well plates with a density of 5 x 10^3 cells/well, treated with varying concentrations of 1–10 mM NaBu or 50–1000 nM TSA, and then incubated under culture conditions for 24 and 48 h, after which 1 mg/ml MTT was added to each well and incubated for a further 4 h. Purple formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and the absorbance of the solution was measured spectrophotometrically at 540 nm using a plate reader (BioTek Instruments, USA).

Semi-quantitative RT-PCR

Total RNA was isolated by phenol-chloroform extraction. RNA (1 µg) was taken for DNase treatment and was reverse transcribed to cDNA by using a RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific, USA). PCR amplification was conducted using primers specific for SLC2A3, 5'-TCCCCTCCGCTGCTCACTATTT-3' (sense), 5'-ATCTCCATGACGCCGTCCTTTC-3' (antisense), GAPDH, 5'-AGGTCGGAGTCAACGGATTTG-3' (sense), and 5'-GTGATGGCATGGACTGTGGT-3' (antisense). The amplification products were electrophoresed on 1% agarose gels and visualized using the ImageQuantTM 4000 image analyzer (GE Healthcare, Life Sciences). The intensities of *SLC2A3* bands were quantitated in relation to GAPDH bands amplified from the same cDNA.

Western blot analysis

Cell extracts were prepared with RIPA lysis buffer containing protease inhibitor (Thermo Scientific, USA). Whole-cell lysates were centrifuged at 14,000 g for 15 min, and protein samples were quantified using the BCA assay kit (iNtRON Biotechnology, Korea). Proteins (30 µg) were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred onto PVDF membranes. Skim milk powder (5% [w/v]) in Tris buffered saline with Tween-20 (TBST) was used to block the membranes for 1 h 30 min at room temperature. The primary antibodies (1:10,000 dilution) rabbit polyclonal anti-GLUT3 (Sigma, USA) or mouse monoclonal anti-actin (1:40,000; Booster, USA) were added and incubated for 20 h at 4 ºC, and then the membranes were washed three times with TBST. Membranes were then incubated with goat anti-rabbit or anti-mouse antibodies for 1 h at room temperature and then washed three times. Chemiluminescence signals were detected by using an ECL kit (Merck, USA). The protein bands were analyzed using ImageJ software.

Cell transfection

When the cells reached 50%–60% confluency, HCT116-BR cells were transfected with 30 nM SLC2A3-siRNAs and negative control siRNA (Ambion, USA). After 48 h of transfection, the expression of the *SLC2A3* gene and GLUT3 protein were evaluated by using semi-quantitative RT-PCR and western blot analysis, respectively.

Data Analysis

Quantitative data were represented as mean ± standard deviation (S.D.) and were statistically analyzed in triplicate. Significant differences between the two groups were analyzed with the Student's t-test (two tailed). In comparisons with the untreated control, differences were considered significant at $*p < 0.05$ and $**p < 0.01$. In comparisons between the HCT116-PT and HCT116-BR cells, $\#p < 0.05$ was considered significant.

Results

Long-term exposure to butyrate causes resistance

In the present study, resistance to butyrate was evaluated by MTT reduction assay. NaBu treatment showed the susceptibility of HCT116-PT cells in that their cell viability was clearly decreased when they were exposed to NaBu for 48 h. Our results illustrated that treatment with 1–5 mM NaBu for 24 h and with all

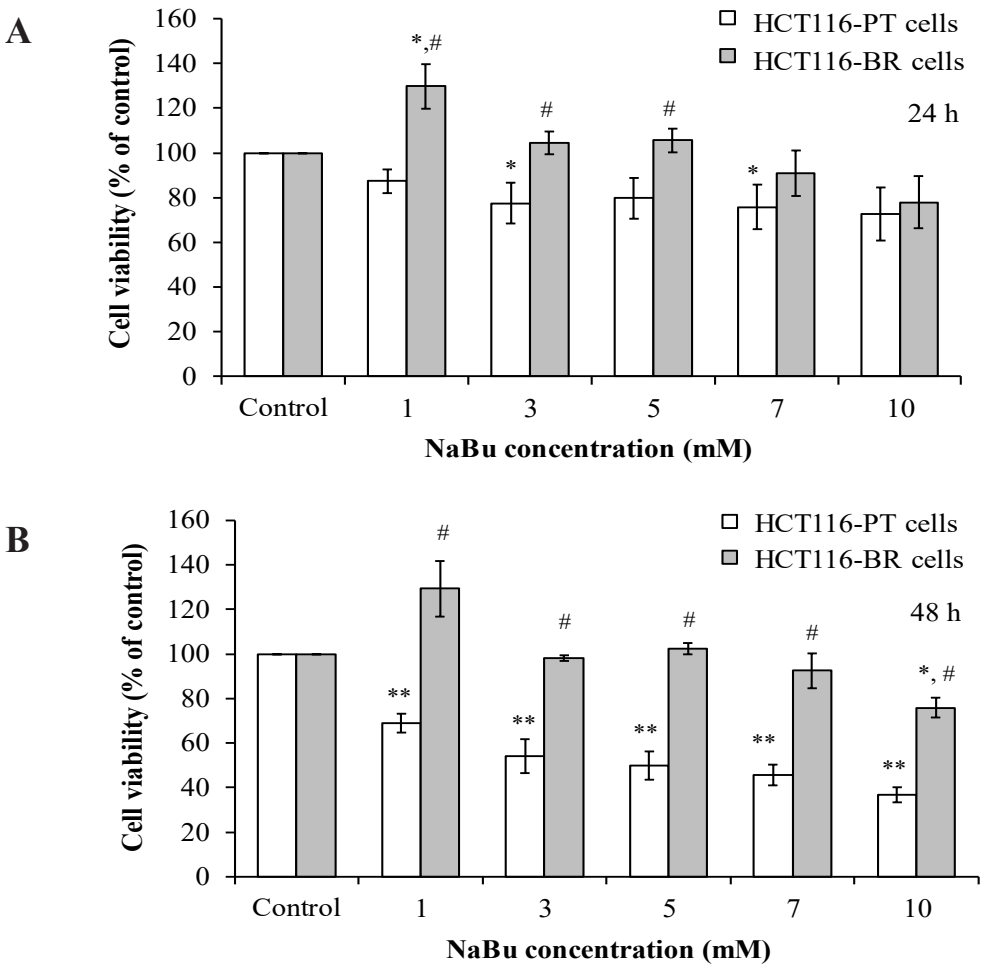

Figure 1. Chronic Exposure to NaBu Causes Resistance to Butyrate-Induced Cell Death. HCT116-PT and HCT116-BR cells were treated with 0–10 mM NaBu for 48 h (B). Cell viability was evaluated using the MTT reduction assay and presented as a percentage of the control. The values are mean ± S.D. (n = 3). *p < 0.05 **p < 0.01 indicate significant differences compared with the untreated control. #p < 0.05 indicates a significant difference in cell viability between the two cell types.

concentrations for 48 h showed a significant difference ($p < 0.05$) in the cell viability of HCT116-PT and HCT116-BR cells. The corresponding concentrations that resulted in 50% cytotoxicity (IC_{50} values) are shown in Table 1. Unexpectedly, treatment with 1 mM butyrate increased the cell viability of HCT116-BR cells by over 120% (Figure 1). Comparative chemosensitivity indicated that HCT116-BR cells were more tolerant to butyrate-induced cell death than HCT116-PT cells and confirmed that the establishment of butyrate-resistant CRC cells by gradually increasing the concentrations of butyrate and further culturing with media containing 3 mM butyrate for 3 months caused the acquired resistance.

Butyrate-mediated acquisition of TSA resistance

TSA is an HDAC inhibitor that exhibits anti-tumor mechanisms similar to those of butyrate. We hypothesized that butyrate-insensitive CRC cells might resist other HDAC inhibitors. Evaluation of butyrate-induced resistance to TSA was performed by using the MTT reduction assay. HCT116-PT and HCT116-BR cells were treated with various concentrations (0–1000 nM) of TSA for 24 and 48 h. The results showed that HCT116-PT cells were more sensitive to the effects of TSA. The cell viability of HCT116-PT cells was clearly decreased in a concentration-and time-dependent manner. Treatment with 750 and 1000 nM TSA for 24 h and 48 h showed significant

Table 1. IC_{50} Values of Chemotherapeutics Agents in HCT116-PT and HCT116-BR Cells

Chemotherapeutic agents	Incubation time	HCT116-PT	HCT116-BR
NaBu (mM)	24 h	35.6	ND
	48 h	5.02	ND
TSA A (nM)	24 h	925.5	2892.75
	48 h	624.75	1109

IC_{50}, the concentrations inhibiting the cell growth by 50%; ND, Not detected

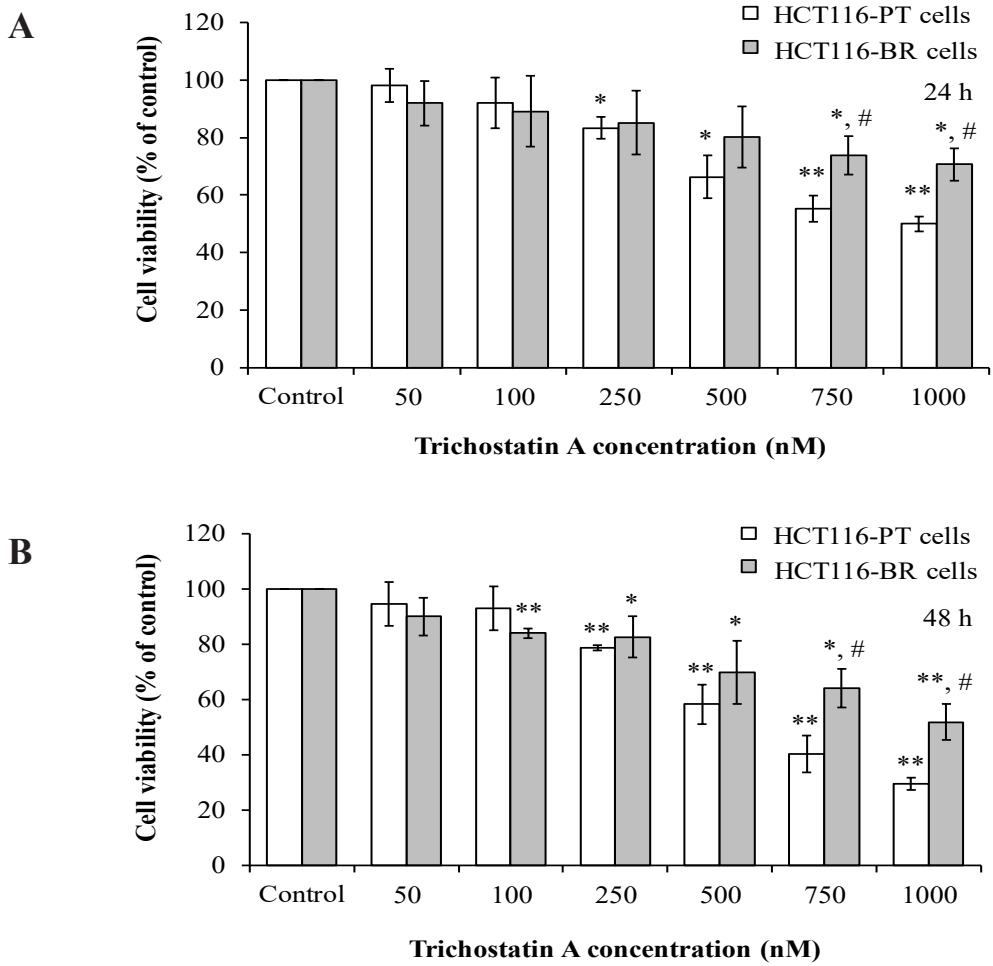

Figure 2. Butyrate-mediated Acquisition of TSA Resistance, HCT116-PT and HCT116-BR Cells were Treated with 50–1000 nM TSA for 24 h (A) and 48 h (48) and then Evaluated by MTT Reduction Assay, and the Results are Presented as a Percentage of the Control. Values represent the means ± S.D. (n = 3). *$p < 0.05$ **$p < 0.01$ indicate significant differences compared with the untreated control. #$p < 0.05$ indicates a significant difference in cell viability between the two cell types.

differences ($p < 0.05$) between the cell viability of parental and butyrate-resistant cells (Figure 2). The respective IC_{50} values are shown in Table 1. These data suggest that acquired resistance to butyrate is accompanied by TSA resistance.

Butyrate-induced overexpression of SLC2A3 mRNA and GLUT3 protein

Preliminary, *SLC2A3* expression was investigated in butyrate-insensitive cells for comparison with HCT116-PT cells. *SLC2A3* mRNA was induced in HCT116-BR cells cultured in DMEM containing 3 mM NaBu but was not found in the parental cells. The investigation of *SLC2A3* mRNA expression during butyrate induction indicated that it was initially detected when the cells encountered 1 mM NaBu (Figure 3A). The level of *SLC2A3* and GLUT3 upregulation was determined using semi-quantitative RT-PCR and western blotting, respectively. The results showed that gene expression of *SLC2A3* in HCT116-PT cells was suppressed, slightly affecting the expression of GLUT3. Long-term exposure to butyrate revealed that upregulation of the *SLC2A3* gene was significantly increased by more than 20 fold ($p < 0.01$), and that of GLUT3 was elevated by approximately 2 fold ($p < 0.05$) in HCT116-BR cells when compared with parental cells (Figure 3B and C).

Knockdown of SLC2A3 sensitizes cells to the effects of TSA

The correlation of GLUT3 upregulation and resistance to TSA was examined using pre-designed siRNAs (Ambion, USA). The efficiency of *SLC2A3* silencer was preliminarily validated for selection of a high-affinity siRNA. HCT116-BR cells at 50%–60% confluence were treated with 30 nM of scrambled (-) siRNA, *SLC2A3* siRNA1 (ID18786), or *SLC2A3* siRNA2 (ID122510) under optimized conditions, followed by semi-quantitative RT-PCR and western blotting. The results showed that *SLC2A3* siRNA2 (ID122510) was more efficient (Figure 4), and it was subsequently selected for evaluation of chemosensitivity by MTT reduction assay. We found that *SLC2A3*-silenced HCT116-BR cells were susceptible to 100-500 nM TSA ($p < 0.05$) when compared with scramble (Figure 5). The IC_{50} values of TSA in HCT116-BR and *SLC2A3*-silenced HCT116-BR experiments were 913.75 and 734.25 nM, respectively. These studies indicated that interference with GLUT3

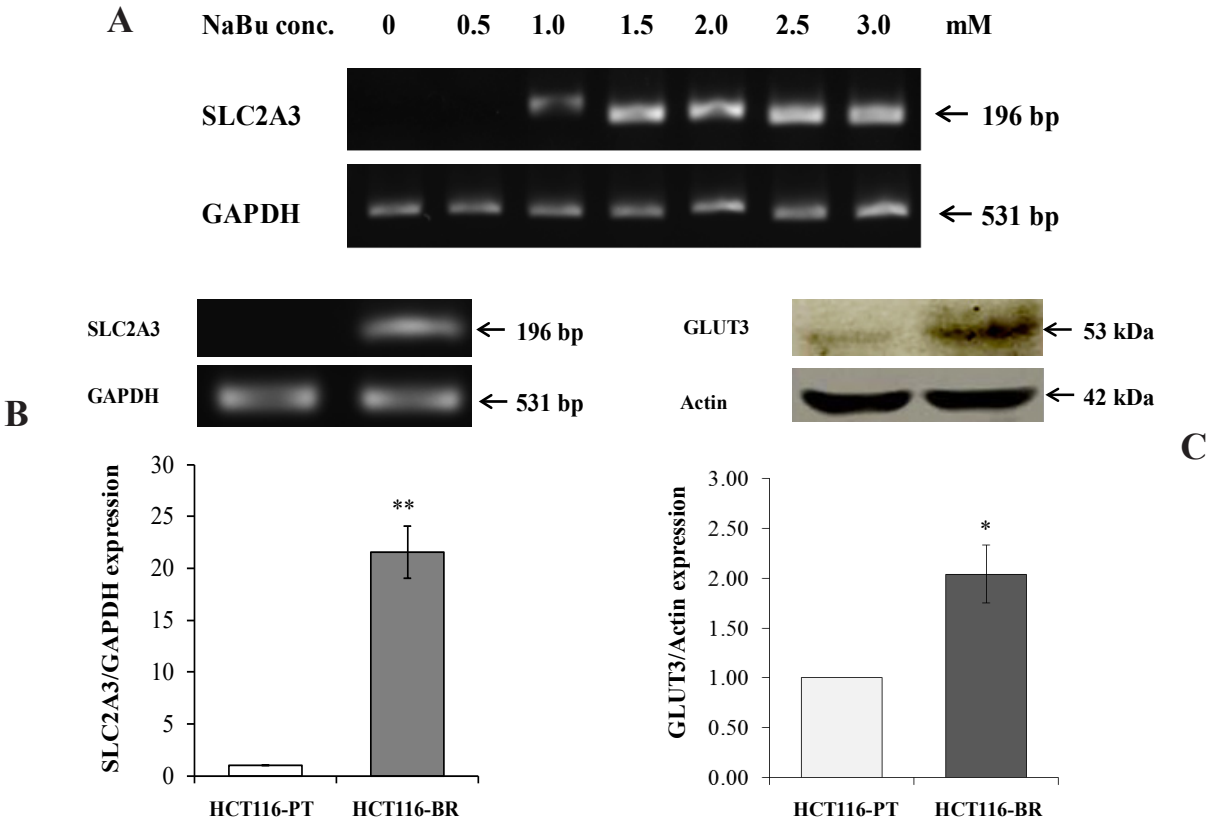

Figure 3. Upregulation of the SLC2A3 Gene in Butyrate-resistant CRC Cells, Butyrate Induction of SLC2A3 During Establishment of an HCT116-BR Cell Model (A). Semi-quantitative RT-PCR revealed inducible expression of the SLC2A3 gene (B), and western blotting showed overexpression of GLUT3 with butyrate resistance (C). Values represent the means ± S.D. from three separate experiments, which represent relative levels normalized to an internal control. *p < 0.05 **p < 0.01 indicate significant differences compared with parental cells.

expression by siRNA (ID122510) chemo-sensitized HCT116-BR cells to TSA and suggested that GLUT3 correlated with butyrate-induced resistance to TSA.

Discussion

Chemoresistance usually results from long-term exposure to chemotherapeutic agents (Zhang et al., 2008; Lui et al., 2014) and is still a critical obstacle

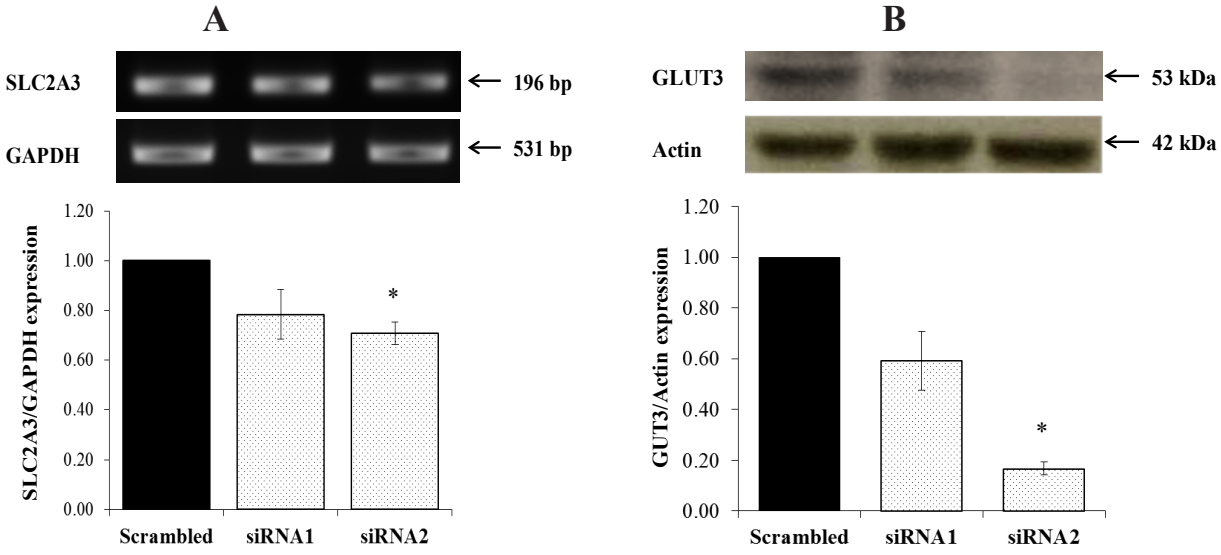

Figure 4. Effects of siRNAs on the Expression of the SLC2A3 Gene (A) and GLUT3 Protein (B). The cells were treated with scrambled, SLC2A3 siRNA1, and siRNA2 for 48 h. The expression of the SLC2A3 gene and GLUT3 were assessed by semi-quantitative RT-PCR and western blot analysis, respectively. Values represent the means ± S.D. from three separate experiments, which represent relative levels normalized to the internal control. *p < 0.05 **p < 0.01 indicate significant differences compared with parental cells.

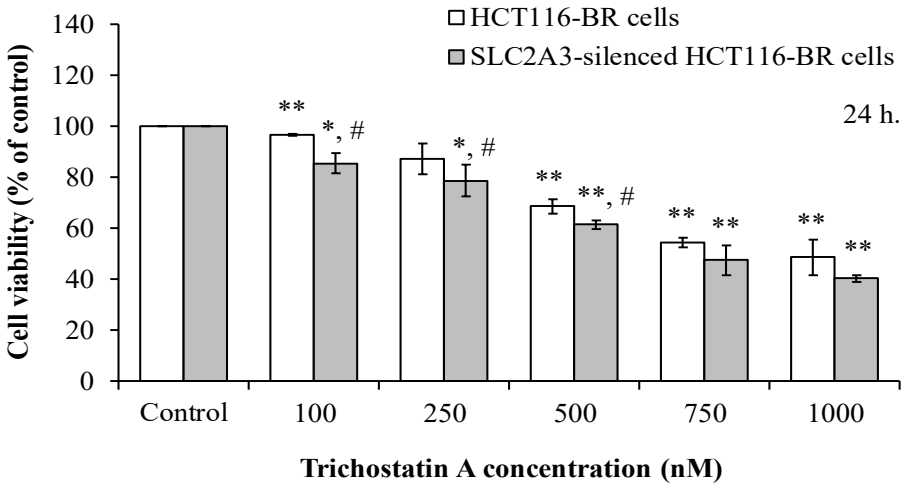

Figure 5. Effects of TSA on the Cell Viability of HCT116-BR and SLC2A3-silenced HCT116-BR Cells. The cells were treated with 100, 250, 500, 750, and 100 nM TSA for 24 h. Cell viability was examined using an MTT reduction assay and is presented as a percentage of the control. Values represent the means ± S.D. (n = 3). *p < 0.05 **p < 0.01 indicate a significant difference from the untreated control. #p < 0.05 indicates a significant difference in cell viability between cell viability of them.

to successful CRC treatment. Our study revealed that the establishment of a chemoresistant CRC cell model by chronic activation with butyrate induced resistance to butyrate-mediated cytotoxicity. Moreover, we found that treatment with 1 mM NaBu caused a greater increase in cell viability than the other concentrations tested (Figure 1). It is possible that our induction procedures more potently triggered certain mechanisms for neutralizing toxic effects or utilizing butyrate to produce the energy for cell activities when cells encountered a concentration lower than 3 mM NaBu. Normally, butyrate exerts its effects on the normal growth of colonocytes via upregulation of p-Erk1/2, which has been confirmed in N/LCM460 cells, but exhibits toxic effects on cancer cells by p-Erk1/2 downregulation (Zeng et al., 2017). It has been reported that long-term exposure to butyrate induced upregulation of p-Erk1/2 in HCT116-BR cells (Kang et al., 2016). It is possible that surviving cells attempt to adapt their response to butyrate to imitate that of normal colonocytes.

Acquired resistance to butyrate was shown to be accompanied by TSA tolerance that could be detected when HCT116-BR cells were exposed to high concentrations (750 and 1000 nM) of TSA for 24 and 48 h (Figure 2). These findings show that long-term exposure to butyrate is able to induce a resistant potential to an anti-neoplastic agent that exhibits a cytotoxic mechanism similar to that of butyrate. Resistance to TSA in HCT116 carcinoma cells has been reported by Imesch et al. (2009), who demonstrated that TSA-resistant cells established defensive strategies via a decrease in H3 and H4 acetylation and upregulation of antiapoptotic Bcl-xL protein. Recently, it has been reported that butyrate-induced TSA resistance was accompanied by the alteration of several molecules, including the elevated expression of Bcl-xL protein, Cyclin D and E, the downregulation of Bax and Bim proteins, and changed the G1-S-G2 pattern of the cell cycle (Kang et al., 2017).

Our results revealed that resistance to HDAC inhibitors was associated with the expression of *SLC2A3* gene which was initially induced by 1 mM NaBu and constitutively expressed during the establishment of the BR cell model (Figure 3A). These results might indicate an involvement of *SLC2A3* in drug tolerance. Investigation by semi-quantitative RT-PCR and western blotting showed that *SLC2A3* was significantly overexpressed by approximately 21 fold ($p < 0.01$), while GLUT3 was highly expressed by 2 fold ($p < 0.05$) in HCT116-BR cells (Figure 3B and 3C), which was a glucose transport-dependent response to butyrate stress conditions. It has been reported that GLUT3 expression was activated by hypoxia-inducible factors (HIFs), the transcription factors responsible for hypoxia (Lauer et al., 2019). Butyrate exerts its anti-tumor effects via properties of an HDAC inhibitor. It has been reported that induced upregulation of *SLC2A3* was found in non-small-cell lung cancer (NSCLC) cell lines that were exposed to vorinostat, a hydroxymate HDACi (O'Byrne et al., 2011). Thus, butyrate-induced upregulation of *SLC2A3* and GLUT3 possibly occurs via epigenetic strategies of the HDAC inhibitor. GLUT3 possesses both a higher affinity for glucose and at least a 5-fold greater transport capacity than GLUT1, GLUT2, and GLUT4. Overexpression of GLUT3 might be a response to the high glucose consumption that is necessary for resistance to butyrate toxicity by ramping up ATP production to support the activity of ATP-binding cassette (ABC) transporters and other drug-defensive strategies.

Attempts have been made in the last decade to investigate the correlation between GLUTs and chemoresistance. In the present study, we found that knockdown of *SLC2A3* by siRNA re-sensitized HCT116-BR cells to low concentrations of TSA (Figure 4). Accumulating evidence implicates GLUTs in drug insensitivity. Wang and colleagues (2013) reported that suppression of GLUT1 expression by shRNA caused a decrease in glucose uptake

and induced vulnerability to cisplatin in the head-neck cancer cell line Cal27. Upregulation of GLUT1 is also associated with 5-FU resistance in colorectal adenocarcinoma HT29 and SW48 cells, and treatment with WZB117, a specific inhibitor of GLUT1, overcomes 5-FU resistance mediated by diminished glucose uptake (Liu et al., 2014). Zhuang et al. (2018) demonstrated that siRNA-mediated silencing of *SLC2A3* transcription promoted apoptosis and chemosensitivity to vincristine in acute myeloid leukemia cells. Chemoresistance and aggressiveness have been described by the Warburg effect, which is characterized by high glucose consumption, accelerated glycolytic activity and rapid ATP production (Bhattacharya, Omar, and Soong, 2016; Icard et al., 2018). Recently, it has been suggested that the expression of GLUT3 is the key to withstanding energy stress (Dai et al. 2020). Therefore, disturbance in glucose uptake directly affects the glycolytic pathway and intracellular ATP levels, which contribute to the ineffective drug-fighting strategies of malignant cells. These findings revealed that interruption by *SLC2A3* siRNA sensitized HCT116-BR cells to TSA-induced cytotoxicity and imply a correlation between GLUT3 and butyrate-mediated acquisition of TSA resistance.

Long-term exposure to butyrate mediated the acquisition of TSA resistance, which was associated with overexpression of GLUT3, implying glucose addiction and resistance to butyrate and TSA. GLUT3 knockdown increases TSA sensitivity. Therefore, GLUT3 might play a crucial role in resistance to anti-tumor HDAC inhibitors and maybe a candidate molecule for predicting chemoresistance or used as a molecular target for drug development.

Author Contribution Statement

All authors contributed equally in this study.

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Conflict of interest

The authors declare no conflicts of interest.

References

Adekola K, Rosen ST, and Shanmugam M (2012). Glucose transporters in cancer metabolism. *Curr Opin Oncol*, **24**, 650-4.

Barron CC, Bilan PJ, Tsakiridis T, Tsiani E (2016). Facilitative glucose transporters: Implications for cancer detection, prognosis and treatment. *Metab Clin Exp*, **65**, 124-39.

Bhattacharya B, Omar MFM, Soong R (2016). The Warburg effect and drug resistance. *Br J Pharmacol*, **173**, 970-9.

Cantor JR, Sabatini DM (2012). Cancer Cell Metabolism: One Hallmark, Many Faces. *Cancer Discov*, **2**, 881-98.

Cao X, Fang L, Gibbs S, et al (2007). Glucose uptake inhibitor sensitizes cancer cells to daunorubicin and overcomes drug resistance in hypoxia. *Cancer Chemother Pharmacol*, **59**, 495–505.

Dai W, Xu Y, Mo S, et al (2020). GLUT3 induced by AMPK/CREB1 axis is key for withstanding energy stress and augments the efficacy of current colorectal cancer therapies. *Signal Transduct Target Ther*, **5**, 177.

Donohoe DR, Holley D, Collins LB, et al (2014). A Gnotobiotic Mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discov*, **4**, 1387-97.

Doyle VC (2007). Nutrition and colorectal cancer risk: a literature review. *Gastroenterol Nurs*, **30**, 178-82.

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. *Cell*, **144**, 646-74.

Icard P, Shulman S, Farhat D, et al (2018). How the Warburg effect supports aggressiveness and drug resistance of cancer cells?. *Drug Resist Updat*, **38**, 1-11.

Imesch P, Dedes KJ, Furlato M, Fink D, Fedier A (2009). MLH1 protects from resistance acquisition by the histone deacetylase inhibitor trichostatin A in colon tumor cells. *Int J Oncol*, **35**, 631-40.

Jiang T, Zhou M-L, Fan J (2018). Inhibition of GLUT-1 expression and the PI3K/Akt pathway to enhance the chemosensitivity of laryngeal carcinoma cells in vitro. *OncoTargets Ther*, **11**, 7865-72.

Kang HR, Choi HG, Jeon CK, Lim S, Kim SH (2016). Butyrate-mediated acquisition of chemoresistance by human colon cancer cells. *Oncol Rep*, **36**, 1119-26.

Khonthun C, Saikachain N, Popluechai S, Kespechara K, Hiranyakas A (2020). Microarray analysis of gene expression involved in butyrate-resistant colorectal carcinoma HCT116 cells. *Asian Pac J Cancer Prev*, **21**, 1739-46.

Kuang R, Jahangiri A, Mascharak S, et al (2017). GLUT3 upregulation promotes metabolic reprogramming associated with antiangiogenic therapy resistance. *JCI Insight*, **2**, e88815.

Lauer V, Grampp S, Platt J, et al (2019). Hypoxia drives glucose transporter 3 expression through hypoxia-inducible transcription factor (HIF)–mediated induction of the long noncoding RNA NICI. *J Biol Chem*, **295**, 4065-78.

Lee J-W, Yang DH, Park S, et al (2018). Trichostatin A resistance is facilitated by HIF-1α acetylation in HeLa human cervical cancer cells under normoxic conditions. *Oncotarget*, **9**, 2035-49.

Liu W, Fang Y, Wang X-T, et al (2014). Overcoming 5-Fu resistance of colon cells through inhibition of Glut1 by the specific inhibitor WZB117. *Asian Pac J Cancer Prev*, **15**, 7037-41.

Ma L, Zong X (2020). Metabolic symbiosis in chemoresistance: refocusing the role of aerobic glycolysis. *Front Oncol*, **10**, 5.

Mo L, Chen Q, Yang Y, Rui X, Gu J (2016). High expression of GLUT1 and GLUT3 correlate with neoadjuvant chemotherapy ineffectiveness breast cancer patients. *Int J Clin Exp Pathol*, **9**, 9555-61.

O'Byrne KJ, Baird AM, Kilmartin L, et al (2011). Epigenetic regulation of glucose transporters in non-small cell lung cancer. *Cancers (Basel)*, **3**, 1550-65.

Park Y, Hunter DJ, Spiegelman D, et al (2005). Dietary fiber

intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. *JAMA*, **294**, 2849-57.
Pericleous M, Mandair D, Caplin, ME (2013). Diet and supplements and their impact on colorectal cancer. *J Gastrointest Oncol*, **4**, 409-23.
Rawla P, Sunkara T, Barsouk A (2019). Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol*, **14**, 89-103.
Regna N, Reilly CM (2014). Isoform-selective HDAC inhibition in autoimmune disease. *J Clin Cell Immunol*, **5**, 207.
Serpa J, Caiado F, Carvalho T, et al (2010). Butyrate-rich colonic microenvironment is a relevant selection factor for metabolically adapted tumor cells. *J Biol Chem*, **285**, 39211-23.
Shen Z, Liao X, Shao Z, et al (2019). Short-term stimulation with histone deacetylase inhibitor trichostatin a induces epithelial-mesenchymal transition in nasopharyngeal carcinoma cells without increasing cell invasion ability. *BMC Cancer*, **19**, 262.
Wang B, Zou Q, Sun M, et al (2014). Reversion of trichostatin A resistance via inhibition of the Wnt signaling pathway in human pancreatic cancer cells. *Oncol Rep*, **32**, 2015-22.
Wang Y, Hu P-C, Ma Y-B, et al (2016). Sodium butyrate-induced apoptosis and ultrastructural changes in MCF-7 breast cancer cells. *Ultrastruct Pathol*, **40**, 200-4.
Wang Y-D, Li S-J, Liao J-X (2013). Inhibition of glucose transporter 1 (GLUT1) chemosensitized head and neck cancer cells to cisplatin. *Technol Cancer Res Treat*, **12**, 525-35.
Wang T, Ning K, Lu T-X, Hua D (2017). Elevated expression of TrpC5 and GLUT1 is associated with chemoresistance in colorectal cancer. *Oncol Rep*, **37**, 1059-65.
Wang W, Fang D, Zhang H, et al (2020). Sodium Butyrate Selectively Kills Cancer Cells and Inhibits Migration in Colorectal Cancer by Targeting Thioredoxin-1. O*nco Targets Ther*, **13**, 4691-704.
Xu K, Dai X-L, Huang HC, Jiang Z-F (2011). Targeting HDACs: A Promising Therapy for Alzheimer's Disease. *Oxid Med Cell Longev*, **2011**, 143269.
Zeng H, Taussig DP, Cheng W-H, Johnson LK, Hakkak R (2017). Butyrate inhibits cancerous HCT116 colon cell proliferation but to a lesser extent in noncancerous NCM460 colon cells. *Nutrients*, **9**, 25.
Zhang H, Zhao X, Liu H, Jin H, Ji Y (2019). Trichostatin A inhibits proliferation of PC3 prostate cancer cells by disrupting the EGFR pathway. *Oncol Lett*, **18**, 687-3.
Zhuang Y, Zhao J, Xu X, Bi L (2018). Downregulation of GLUT3 promotes apoptosis and chemosensitivity of acute myeloid leukemia cells via EGFR signaling. *Arch Iran Med*, **21**, 73-8.