Introduction

Colorectal cancer (CRC) is the most frequently diagnosed cancer (Rawla et al., 2019), but the critical obstacle in its treatment is still the recurrence of the tumor during long-term survival after treatment. Recurring tumors are endurable to radiation therapy (Tang et al., 2018) and chemotherapy (Zeng, 2017), and are associated with poor prognosis and ineffective clinical outcomes. Currently, cancer stem cells and cancer stem-like cells are often regarded as the target for cancer monitoring. However, the different patterns of their transcriptomic profiling is still unclear. Objective: This study aims to illustrate the transcriptomic profile of CSCs and butyrate-resistant colorectal carcinoma cells (BR-CRCs), by comparing them with parental colorectal cancer (CRC) cells in order to identify distinguishing transcription patterns of the CSCs and BR-CRCs. Methods: Parental CRC cells HCT116 (HCT116-PT) were cultured and induced to establish the butyrate resistant cell model (HCT116-BR). Commercial enriching of the HCT116-CSCs were grown in a tumorsphere suspension culture, which was followed firstly by the assessment of butyrate tolerance using MTT and PrestoBlue. Then their gene expression profiling was analyzed by microarray. Results: The results showed that both butyrate-resistant HCT116 cells (HCT116-BR) and HCT116-CSCs were more tolerant a butyrate effects than HCT116-PT cells. Differentially expressed gene profiles exhibited that IFI27, FOXO1, PRF1, and SLC2A3 genes were increasingly expressed in CSCs, and were dramatically overexpressed in HCT116-BR cells when compared with HCT116-PT cells. Moreover, PKIB and LOC399959 were downregulated both in HCT116-CSCs and HCT116-BR cells. Conclusion: Our findings shed light on the transcriptomic profiles of chemoresistant CRC cells. This data should be useful for further study to provide guidelines for clinical prognosis to determine the guidelines for CRC treatment, especially in patients with chemoresistance and designing novel anti-neoplastic agents.

Keywords: Butyrate- chemoresistance- colorectal cancer- cancer stem cells

Microarray Analysis of Gene Expression Involved in Butyrate-Resistant Colorectal Carcinoma HCT116 Cells

Chakkraphong Khonthun¹, Nongluk Saikachain¹, Siam Popluechai²³, Kongkiat Kespechara⁴, Art Hiranyakas⁵, Metawee Srikummool¹, Damratsamon Surangkul¹*

Abstract

Background: Resistance to chemotherapeutic agents is usually found in cancer stem cells (CSCs) and cancer stem-like cells that are often regarded as the target for cancer monitoring. However, the different patterns of their transcriptomic profiling is still unclear. Objective: This study aims to illustrate the transcriptomic profile of CSCs and butyrate-resistant colorectal carcinoma cells (BR-CRCs), by comparing them with parental colorectal cancer (CRC) cells in order to identify distinguishing transcription patterns of the CSCs and BR-CRCs. Methods: Parental CRC cells HCT116 (HCT116-PT) were cultured and induced to establish the butyrate resistant cell model (HCT116-BR). Commercial enriching of the HCT116-CSCs were grown in a tumorsphere suspension culture, which was followed firstly by the assessment of butyrate tolerance using MTT and PrestoBlue. Then their gene expression profiling was analyzed by microarray. Results: The results showed that both butyrate-resistant HCT116 cells (HCT116-BR) and HCT116-CSCs were more tolerant a butyrate effects than HCT116-PT cells. Differentially expressed gene profiles exhibited that IFI27, FOXO1, PRF1, and SLC2A3 genes were increasingly expressed in CSCs, and were dramatically overexpressed in HCT116-BR cells when compared with HCT116-PT cells. Moreover, PKIB and LOC399959 were downregulated both in HCT116-CSCs and HCT116-BR cells. Conclusion: Our findings shed light on the transcriptomic profiles of chemoresistant CRC cells. This data should be useful for further study to provide guidelines for clinical prognosis to determine the guidelines for CRC treatment, especially in patients with chemoresistance and designing novel anti-neoplastic agents.

Keywords: Butyrate- chemoresistance- colorectal cancer- cancer stem cells

Asian Pac J Cancer Prev, 21 (6), 1739-1746
by bacterial fermentation in the colon, and acts as an energy source for colonocytes and plays a role in colonic homeostasis. Like other HDAC inhibitors, it inhibits cell proliferation (Zeng et al., 2017) and induces apoptosis in cancerous cells (Ruenmele et al., 2003; Pajak et al., 2007; Roy et al., 2009). It has been suggested as the anti-proliferating agent with slight side effects for CRC treatment, and chronic exposure of it causes butyrate insensitivity (Kang et al., 2016). It has also been demonstrated that butyrate-rich colonic microenvironment contributes to tumor aggressiveness, resulting from the adaptation of subpopulation of butyrate-challenging CRCs (Serpa et al., 2010).

CSCs and butyrate-resistant colorectal carcinoma cells (BR-CRCs) are insensitive to chemotherapeutic drugs, while still being barriers to CRC treatment. In butyrate-rich condition, it seems that BR-CRCs express cancer stem-like features, and it has been reported that acquired butyrate resistance also overcomes anticancer effects of other chemotherapeutic drugs, such as paclitaxel, 5-fluorouracil, doxorubicin and trichostatin A (Kang et al., 2016). The in vivo studies demonstrated that butyrate resistance related to tumorigenicity of the CRC cells, were associated with aggressive phenotypes (Serpa et al., 2010). Although they are tolerant to antineoplastic agents, their gene expression profiles are still not fully understood. In this study, we established a BR-CRCs model from colorectal carcinoma cells, and the transcriptome profiling was assessed by comparing differentially expressed genes of CSCs, BR-CRCs, and CRCs. The profiles of the gene expressions will provide an insight to the transcriptomic patterns and candidate genes that relates to drug resistance for monitoring the CRC treatment and effective therapy.

Materials and Methods

Cell culture

HCT116 colon cancer cell lines were obtained from American Type Culture Collection (ATCC). The cells were grown as monolayer in McCoy’s 5A with L-glutamine (Corning, USA) supplemented with 10% heat inactivated fetal bovine serum (HI-FBS) and 100U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B, then cultured at 37%C in a humidified atmosphere of 95% air and 5% CO2.

The parental HCT116 (HCT116-PT) cells were cultured in complete media, with a gradually increasing concentration of sodium butyrate (NaBu) for each 2 passages (Fung et al., 2009) by adding of 0.5-5.0 mM NaBu finally, the surviving cells were maintained with a media containing 3 mM NaBu to produce a butyrate-resistant HCT116 cell model (HCT116-BR).

The HCT116-CSCs were purchased from Promab Biotechnologies (Promab Biotechnologies, USA). Tumorspheres were grown in an ultra-low binding plate and cultured with Cancer Stem Premium™ media (Promab Biotechnologies, U.S.A.) containing 100U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B. CSCs aggregated to form the tumorsphere which were visible by an inverted light microscope, followed by subcultivation that was performed every 8-10 day.

Assessment of butyrate tolerance by MTT and PrestoBlue assay

To confirm the butyrate-resistant ability, cell viability was assessed by MTT reduction assay. HCT116-PT and HCT116-BR cells were seeded in a 96-well plate with a density of 5x104 cells/well, and incubated for sufficient attachment before NaBu treatment. The HCT116-PT cells were treated with various concentrations of 0, 1, 3, 5, 7, 10 mM NaBu (Sigma Aldrich, Switzerland) for 24 and 48 h. McCoy’s 5A containing 3 mM NaBu was used for HCT116-BR cell seeding followed by NaBu treatments, while the control group was exposed to 3 mM NaBu for the same incubation period as the HCT116-PT cells, 1 mg MTT (Ameresco, USA) was added to each well, and was further incubated for 4 h under a culture conditions. Purple formazan crystals were determined using a spectrophotometrically at 540 nm. The PrestoBlue assay was used to assess the butyrate tolerance of HCT116-CSCs. The cells were seeded in a 96-well plate with a density of 5x104 cells/well and treated with the same NaBu concentrations and incubation period. The PrestoBlue™ Reagent (Invitrogen, USA) was added into the culture media and incubated for 2 h, then the fluorescence was measured using a microplate reader (BioTek, USA) at an excitation of 535 and an emission of 615 nm.

Microarray analysis

The expression of human genes was analyzed using a microarray analysis to simultaneously assess the mRNA expression. Cell samples of HCT116-PT, HCT116-BR cells, and HCT116-CSCs were sent to Macrogen, Inc (Macrogen, South Korea). Global gene expression profiling was assessed by using microarray analyzer (Illumina, USA). Total RNA was amplified and purified using TargetAmp-Nano Labeling Kit for Illumina Expression BeadChip (EPICENTRE Biotechnologies, USA) to yield biotinylated cRNA, 200 ng of the total RNA was reverse-transcribed to cDNA using a T7 oligo(dt) primer and labeled with biotin-NTP. Labeled cDNA were hybridized to each Human HT-12 v4.0 Expression BeadChip (Illumina, USA). Signals were detected by using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, UK). Arrays were scanned with an Illumina bead array Reader confocal scanner. Raw data were extracted using the software provided by the manufacturer (Illumina GenomicStudio v2011.1 (Gene Expression Module v1.9.0)). The fold change of gene expressions was considered in HCT116-CSCs vs HCT116-PT cells and HCT116-BR vs HCT116-PT cells. Gene selection was based on the top 10 rankings of the increased fold changes of gene expressions in HCT116-CSCs and HCT116-BR cells when compared with HCT116-PT cells that clearly indicated their genetic features.

Data analysis

Cell viability values are represented as mean ± S.M.E, while statistical differences between the two groups were determined with the Student’s t test. Significance is when the p value is less than 0.05. Data form the microarray was exhibited as the relative gene expression of HCT116-CSCs.
vs HCT116-PT cells and HCT116-BR vs HCT116-PT cells, which were listed in descending order to study their transcriptomic profiling.

**Results**

The effect of NaBu on the cell viability of HCT116-CSCs, HCT116-BR, and HCT116-PT cells

To assess the effect of NaBu on cell viability, HCT116-PT, HCT116-BR cells, and CSCs were treated with different concentrations of NaBu (0, 1, 3, 5, 7, and 10 mM) for 24 and 48 h. The survival of the HCT116-PT and HCT116-BR cells were evaluated by an MTT reduction assay, and Butyrate tolerance of CSCs was investigated using the PrestoBlue assay. The results showed that NaBu decreased cell viability of the HCT116-PT cells and CSCs in concentration and a time-dependent manner, while the endurance of butyrate increased in the HCT116-BR cells (Figure 1A and 1B).

Different gene expression of HCT116-PT/CSCs and HCT116-BR/HCT116-PT cells

Transcriptomic profiling was obtained by microarray analysis. The total RNA of the HCT116-PT, HCT116-BR cells, and HCT116-CSCs were reverse-transcribed to cDNA and subsequently hybridized to each Human HT-12 v4.0 Expression Beadchip (Illumina, Inc., San Diego, USA). The levels of gene expression were calculated and represented as a fold change of expression of HCT116-CSCs vs HCT116-PT cells and HCT116-BR cells vs HCT116-PT cells. The values indicated up- or downregulated genes.

| Gene symbol | Gene ID | Relative expression | Definition |
|-------------|---------|---------------------|------------|
| **Upregulated genes** | | | |
| CEACAM1 | 634 | 5.611914 | Carcinoembryonic antigen-related cell adhesion molecule 1 |
| IFI27 | 3429 | 5.421592 | Interferon alpha-inducible protein 27 |
| FOXLQ1 | 94234 | 5.378125 | Forkhead box Q1 |
| PRF1 | 5551 | 3.659196 | Perforin-1 |
| SNTB1 | 6641 | 3.631548 | Syntrophin Beta 1 |
| FGFBP1 | 9982 | 3.210627 | Fibroblast growth factor binding protein 1 |
| ZBED2 | 79413 | 3.147975 | Zinc finger BED-type containing 2 |
| H19 | 283120 | 3.101951 | non-coding RNA |
| FSTL5 | 56884 | 2.991059 | Follistatin like 5 |
| SLC2A3 | 6515 | 2.826233 | Facilitated glucose transporter member 3 |
| **Downregulated genes** | | | |
| NR4A3 | 8013 | 2.544763 | Nuclear receptor subfamily 4 group A member 3 |
| PKIB | 5570 | 2.312368 | Protein kinase (cAMP-dependent, catalytic) inhibitor beta |
| TRNP1 | 388610 | 2.311719 | TMF1-Regulated Nuclear Protein |
| RGS2 | 5997 | 2.192854 | Regulator of G-Protein Signaling |
| LOC399959 | 399959 | 2.191778 | Homo sapiens hypothetical LOC399959, non-coding |
| STK39 | 27347 | 2.138326 | Serine/Threonine Kinase 39 |
| CYR61 | 3491 | 2.084594 | Cysteine Rich Angiogenic Inducer 61 |
| FER1L4 | 80307 | 2.057106 | Fer-1 like family member 4 |
| MMP7 | 4316 | 2.04973 | Matrix metallopeptidase 7 |
| PPARG | 5468 | 2.039141 | Peroxisome proliferator activated receptor gamma |

Figure 1. The Effect of NaBu on the Cell Viability of HCT116-PT, HCT116-BR Cells and HCT116-CSCs. The cells were treated with different concentrations of NaBu (0, 1, 3, 5, 7, and 10 mM) for 24 (A) and 48 h (B), followed by the cell viability being evaluated using an MTT and PrestoBlue assay then presented as a percentage of control. ($p<0.05$ *significance with HCT116-CSCs and HCT116-PT cells, **significance with HCT116-BR cells and HCT116-CSCs).
Differentially expressed genes of HCT116-CSCs, compared with the HCT116-PT cells

The comparison of global gene expression between HCT116-CSCs and HCT116-PT cells was conducted to assess the gene expression profiling that indicated the intrinsic resistance to butyrate and cancer stemness. The microarray data revealed that the top 10 up-regulated genes that mostly participated in aggressive phenotype and cancer progression, consisting of CEACAM1, IFI27, FOXQ1, PRF1, SNTB1, FGFBP1, ZBED2, H19, FSTL5, and SLC2A3 genes. These could be classified into 3 groups, (1) the metastasis and tumorigenesis-promoting factors containing CEACAM1, IFI27, FOXQ1, and H19, (2) survival molecules; FGFBP1 and SLC2A3, and (3) tumor-associated factors, including PRF1, SNTB1, ZBED2, and FSTL5. The cancer stemness features were associated with slightly decreased expression of PKIB, TRNP1, RGS2, STK39, CYR61, MMP7, and PPAR genes.
which often participated in cell proliferation (Table 1).

**Differentially expressed genes of the HCT116-BR cells, compared with the HCT116-PT cells**

HCT116-BR cells which are more resistant to butyrate effects exhibited the top 10 upregulations of *IFI27*, *BST2*, *SLC2A3*, *KRT13*, *DHR52*, *IF16*, *PRF1*, *PLAC8*, *OLFMI1*, and *FOXQ1* genes. They could be classified as (1) tumor growth and metastasis promoting factors containing *IFI27*, *FOXQ1*, *KRT13*, *IF16*, and *PLAC8* genes, (2) glucose transporter has the *SLC2A3* gene, (3) the chemoresistance-associated molecules comprising the *DHR52* and *BST2* genes, and (4) the *PRF1* and *OLFMI1* genes with unclear functions. Interestingly, *IFI27*, *FOXQ1*, and *SLC2A3* genes were dramatically overexpressed when compared with parental cells. The 10 downregulated genes were *LOC399959*, *MKX*, *ARMC4*, *ACSL5*, *GPR110*, *SCG2*, *PKIB*, *NTSE*, and *AKAP112* which were frequently found in several cancers, but their roles remain unclear (Table 2).

**The gene expression in both HCT116-CSCs and HCT116-BR cells**

According to microarray data, the gene expression profiling showed 4 upregulated genes and 2 downregulated genes that were found in both the HCT116-CSCs and the HCT116-BR cells by varying degree (Figure 2). The upregulations of *IFI27*, *FOXQ1*, *SLC2A3*, and *PRF1* genes in the HCT116-CSCs were detected at 5.42, 5.38, 3.66, and 2.83 folds, respectively whereas extensive overexpressions by 139.86, 97.37, 77.21, and 24.12 folds, respectively were found in the HCT116-BR cells (Table 1). The downregulated genes, *PKIB* and *LOC399959*, were slightly decreased in HCT116-CSCs (Table 1) by approximately 2 fold, while expressions of *LOC399959* and *PKIB* were apparently decreased more than 19 and 7 folds in HCT116-BR cells when compared with HCT116-PT cells (Table 2). These results revealed the correlation of upregulated and downregulated genes which are implicated in intrinsic and acquired resistance of CSCs and BR cells, respectively.

**Discussion**

Tumor recurrence and therapeutic resistance are considered as clinical problems for CRC treatment. The reappearance of tumors leads to poor prognosis and ineffective clinical outcomes. The subpopulation of chemoresistant CRC cells that are distinct from cancer cells in bulky tumors, are often referred to as CSCs and cancer stem-like cells that upregulate the chemoresistance-related molecules by providing drug-fighting mechanisms and contributing to therapeutic failure (Abdullah and Chow, 2013; Murayama and Gotoh, 2019). Butyrate, the anti-neoplastic agent, turns on several anti-cancer mechanisms (Zeng et al., 2017), however, long-term exposure to butyrate mediates the acquisition of resistance to chemotherapeutic drugs (Kang et al., 2016). This study established the butyrate-resistant cell model to reveal the transcriptomic profiles of chemoresistant cells. Our results exhibited that exposure to butyrate caused a decrease in the cell viability of HCT116-PT cells, both in concentration and time-dependent manners, however, our studies found that HCT116-CSCs and HCT116-BR cells were more resistant to the butyrate effects than HCT116-PT cells (Figure 1A and 1B). The intrinsic chemoresistance exist in CSCs (Koren and Fuchs, 2016) whereas acquired chemoresistance is induced with chronic activation with chemotherapeutic drugs. It has been proposed that resistance to butyrate associated with cancer progression (Lazarov and Bordonaro, 2016), and butyrate-rich colonic microenvironment contributes to tumor aggressiveness resulting from the adaptation of subpopulation of butyrate-challenging CRC cells in animal model (Serpa et al., 2010). The assessment of the gene transcription profiles of HCT116-CSCs/HCT116-PT cells and HCT116-BR/HCT116-PT cells revealed that expressions of *IFI27*, *FOXQ1*, *PRF1*, and *SLC2A3* genes were highly increased in HCT116-CSCs, and being dramatically overexpressed in the HCT116-BR cells when compared with the HCT116-PT cells. The analysis of gene expression data also showed downregulations of *PKIB* and *LOC399959* that their levels were slightly decreased in HCT116-CSCs, and greatly declined in HCT116-BR cells (Table 1 and 2).

A slight increase of *IFI27*, *FOXQ1*, *PRF1*, and *SLC2A3* genes in CSCs probably implicate in phenotypes of cancer stem cells that were often quiescent, but being able to exhibit their chemoresistant properties. However, overexpressions of these genes in HCT116-BR cells might be resulted from the establishing of defensive mechanisms against butyrate-induced apoptosis, reflecting cell adaptation responsible for butyrate stress.

Interferon inducible factor27 (IFI27), a hydrophobic mitochondrial protein, has been reported in psoriatic skin (Suomela et al., 2004) and several cancer cells with aggressive phenotypes (Budhu et al., 2007; Li et al., 2015; Wang et al., 2018; Chiang et al., 2019). IFI27 not only induced ovarian tumorigenicity, stemness, but also participated in drug resistance of ovarian cancer cells (Li et al., 2015). Deregulation of *IFI27* expression has been reported in cholangiocarcinoma patients that are associated with poor survival (Chiang et al., 2019). Elevated expression of *IFI27* in HCT116-CSCs might implicate in their cancer stemness characteristics, particularly tumorigenicity. Our results revealed that long-term exposure to butyrate not only established a tolerance to butyrate, but also caused an excess *IFI27* mRNA expression in HCT116-BR cells. Studies by Virag and colleagues (2013) demonstrated that transcriptional activity for *IFI27* gene was increased by acquired resistance to oxaliplatin in IFT-29R cells. Currently, it is well known that the epithelial-mesenchymal transition (EMT) is linked to chemoresistance (Elaskalani et al., 2017; Staalduinen et al., 2018) and cancer progression (Ramos et al., 2017). Study by Li et al (2015) demonstrated that IFI27 promoted EMT and drug resistance. Therefore, excess expression of *IFI27* in HCT116-BR cells might be the defensive mechanisms responsible for chronic butyrate activation and mainly implicated in aggressive phenotypes.

FOXQ1, a transcription factor, acted as the key player in the pathogenesis of tumors (Li et al., 2016)
and correlated with poor prognosis (Zhan et al., 2015). It played a role in EMT in human cancer (Qiao et al., 2011), and has been reported that an upregulation of it was found in the metastatic stage of breast cancer (Zhang et al., 2011), esophageal cancer (Pei et al., 2015), pancreatic cancer (Li et al., 2016) and gastric cancer (Guo et al., 2017). In colorectal cancer, FOXQ1 not only promoted metastatic potential (Abba et al., 2013; Liu et al., 2017) but also enhanced tumorigenicity and tumor growth (Keneda et al., 2010). Elevated expression of FOXQ1 in HCT116-CSCs may be involved in regulation of expression pattern of genes which determine the characteristics of cancer stem cells. Excess FOXQ1 transcription was clearly found in HCT116-BR cells that might participated in gene regulation of butyrate resistance pattern and responding to butyrate actions, especially cell differentiation, and the induction of defensive mechanisms against the butyrate-induced apoptosis, and the HCT116-BR cells may also have metastatic potential that require further investigation.

The accelerated glycolytic activity is the hallmark of cancer that is associated with high glucose consumption by glucose transporters (GLUTs). Our results revealed that expression of the SLC2A3 gene, encoding for GLUT3 protein, was raised in HCT116-CSCs. Christensen et al., (2015) reported that expression of it correlated with OCT4, which is a candidate marker for CSCs and the NANOG and is regulated by genes which participate in the embryonic and cancer stem cell program (Korkola et al., 2006). CSCs reside in a tumor mass where they often encounter hypoxic conditions and glucose deprivation, therefore, it is possible that the SLC2A3 upregulation may respond to the microenvironment of CSCs. The acquisition of resistance to butyrate caused overexpression of SLC2A3 mRNA 77.21 fold that might denote the mechanisms responsible for cell survival, especially chemoresistance, because ATP are necessary for the activity of ATP-binding cassette (ABC) transporters which extrude chemotherapeutic agents. Previous studies showed the correlation between chemoresistance and SLC2A1 (Song et al., 2014; Wang et al., 2017). However, overexpression of SLC2A3 and drug resistance is less well defined. GLUT3 served as the transporter with high affinity responsible for rapid glucose uptake. It is possible that enhanced SLC2A3 expression in HCT116-BR cells were defensive mechanisms, responding to butyrate stress, and ultimately contributed to aggressive phenotypes.

Perforin-1 (PRF1) which is normally found in the granules of cytotoxic T lymphocytes (CTLs) and natural killer cells, plays a role in immune response (Podack and Munson, 2016). Our results revealed that its upregulation could be detected both in HCT116-CSCs and HCT116-BR cells, while its role in cancer and chemoresistance has not yet been identified.

Our results showed the decreased expression of PKIB and LOC399959 in both HCT116-CSCs and HCT116-BR cells. PKIB promoted cell proliferation (Dou et al., 2016), while slight down-regulation of it appeared in the HCT116-CSCs which might participate in the feature of cancer stem cells, but their expression greatly reduced in the HCT116-BR cells. It is possible that a decrease in the PKIB expression is a strategy to reduce cell activity and preserve energy for the survival of CSCs and the downregulation in HCT116-BR cells, may decrease in response to butyrate-induced apoptosis, which leads to butyrate resistance, while LOC399959 is a non-coding RNA, whose function in cancer is still unknown. The previous studies focused on butyrate resistance in adenocarcinoma by using HT29 (Fung et al., 2009) and BCS-T2C2 cells (Silanes et al., 2004), however, HCT116-BR cells have been reported by Kang et al., (2016). Who established the butyrate resistant CRC and maintained in media containing 1.6 mM butyrate which differed our study. They demonstrated that acquired resistance to butyrate led to chemoresistance and did not point to gene expression. Our BR cell model that was induced with 0.5-3.0 mM and maintained with 3.0 mM butyrate has never been found elsewhere.

In conclusion, chemoresistant cells are referred to as CSCs and cancer stem-like cells, so even though they are not identical, CRC treatment is focused on eradicating both of them. The upregulations of the IFI27, FOXQ1, PRF1, and SLC2A3 genes and the downregulation of the LOC399959 and PKIB genes by varying degrees, possibly implicate the resistance to butyrate of the HCT116-CSCs and the HCT116-BR cells. A slight change in the gene expression of the HT116-CSCs may be involved in the CSC niche and chemoresistance, whereas overexpression in the HCT116-BR cells, might result from butyrate-induced cell adaptation or the defensive mechanisms responsible for chronic butyrate activation that may predominantly implicate the aggressive phenotypes. Although transcriptomic patterns of intrinsic and acquired resistance to butyrate are not identical, we expected that either high or overexpression of these genes were useful in selecting and designing novel anti-cancer agents for the effective targeting of CRC therapy.

Acknowledgements

This work was sponsored by Bangkok Hospital Phuket, Phuket. CK was supported by the National Research Council of Thailand (Grant No.2562/3). MS was supported by Naresuan University (Grant No. R2562/B085). The author would like to thank MR. Peter Barton, of Division of International Affairs and Language Development (DIALD) for his editing services of the English in this work.

Statement conflict of Interest

Authors declare no conflict of interest.

References

Abba M, Patil N, Rasheed K, et al (2013). Unraveling the role of FOXQ1 in colorectal cancer metastasis. Mol Cancer Res, 11, 1017-28.

Abdullah LN, Chow EK (2013). Mechanisms of chemoresistance in cancer stem cells. Clin Transl Med, 2, doi: 10.1186/2001-1326-2-3.

Budda A, Chen Y, Kim JW, et al (2007). Induction of a unique gene expression profile in primary human hepatocytes by hepatitis C virus core, NS3 and NS5A proteins.
Carcinogenesis, 28, 1552-60.
Chiang KC, Huang ST, Wu RC, et al (2019). Interferon α-inducible protein 27 is an oncogene and highly expressed in cholangiocarcinoma patients with poor survival. Cancer Manag Res, 11, 1893-05.

Christensen DR, Calder PC, Houghton FD (2015). GLUT3 and PKM2 regulate OCT4 expression and support the hypoxic culture of human embryonic stem cells. Sci Rep, 5, doi: 10.1038/srep17500.

Dou P, Zhang D, Cheng Z, Zhou G, Zhang L (2016). PKIβ promotes cell proliferation and the invasion-metastasis cascade through the PI3K/Akt pathway in NSCLC cells. Exp Biol Med (Maywood), 241, 1911-8.

El Khoury F, Corcos L, Durand S, Simon B, Le Jossic-Corcos C (2018). Acquisition of anticancer drug resistance is partially associated with cancer stemness in human colon cancer cells. Int J Oncol, 49, 2558-68.

Elkaskalani O, Razak NB, Falasca M, Metharom P (2017). Epithelial-mesenchymal transition as a therapeutic target for overcoming chemoresistance in pancreatic cancer. World J Gastrointest Oncol, 9, 37-41.

Fung KYC, Lewanowitsch T, Henderson ST, et al (2009). Proteomic analysis of butyrate effects and loss of butyrate sensitivity in HT29 colorectal cancer cells. J Proteome Res, 8, 1220-7.

Guo J, Yan Y, Yan Y, et al (2017). Tumor-associated macrophages induce the expression of FOXQ1 to promote epithelial-mesenchymal transition and metastasis in gastric cancer cells. Oncol Rep, 38, 2003-10.

Korkola JE, Houldsworth J, Chadalavada, RS, et al (2006). Down-regulation of stem cell genes, including those in a 200-kb gene cluster at 12p13.31, is associated with in vivo differentiation of human male germ cell tumors. Cancer Res, 66, 820-7.

Ibragimova MK, Tsyganov MM, Litviakov NV (2017). Natural product, Vimentin, colon cancer cells. Biochemistry (Mosc), 82, 413-25.

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

Koren E, Fuchs Y (2016). The bad seed: Cancer stem cells and drug resistance to butyrate. Cancer Res, 76, 4953-9.

Korkola JE, Houldsworth J, Chadalavada, RS, et al (2006). Down-regulation of stem cell genes, including those in a 200-kb gene cluster at 12p13.31, is associated with in vivo differentiation of human male germ cell tumors. Cancer Res, 66, 820-7.

Lazarova DL, Bordonaro M (2016). Vimentin, colon cancer progression and resistance to butyrate and other HDACIs. J Cell Mol Med, 20, 989-93.

Li S, Xie Y, Zhang W, et al (2015). Interferon alpha-inducible protein 27 promotes epithelial-mesenchymal transition and induces ovarian tumorigenicity and stemness. J Surg Res, 193, 255-64.

Li Y, Zhang Y, Yao Z, et al (2016). Forkhead box Q1: A key player in the pathogenesis of tumors (Review). Int J Oncol, 49, 51-8.

Liu JY, Wu XY, Wu GN, Liu F-K, Yao X-Q (2017). FOXQ1 promotes cancer metastasis by PI3K/AKT signaling regulation in colorectal carcinoma. Am J Transl Res, 9, 2207-18.

Murayama T, Gotot N (2019). Drug resistance mechanisms of cancer stem-like cells and their therapeutic potential as drug targets. Cancer Drug Resist, 2, 457-70.

Pająk B, Orzechowska A, Gajkowska D (2007). Molecular basis of sodium butyrate-dependent proapoptotic activity in cancer cells. Adv Med Sci, 52, 83-8.

Pei Y, Wang P, Liu H, He F (2015). FOXQ1 promotes epidermoid cancer proliferation and metastasis by negatively regulating CDH1. Biomed Pharmacother, 74, 89-94.

Podack ER, Munson GP (2016). Killing of microbes and cancer by the immune system with three mammalian pore-forming killer proteins. Front Immunol, 7, doi: 10.3389/fimmu.2016.00464.

Qiao Y, Jiang X, Le ST, et al., (2011). FOXQ1 regulates epithelial-mesenchymal transition in human cancers. Cancer Res, 71, 3076-86.

Ramos FS, Wons L, Cavalli IJ, Ribeiro EMSF (2017). Epithelial-mesenchymal transition in cancer: An overview. Integr Cancer Sci Ther, 4, doi: 10.3390/cancers10020052.

Rawla P, Sunkara T, Barsouk A (2019). Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Gastroenterol Rev, 14, 89-103.

Roy M-J, Dionne S, Marx G, et al (2009). In vitro studies on the inhibition of colon cancer by butyrate and carnosine. Nutrition, 25, 1193-01.

Ruemmele FM, Schwartz S, Seidman EG, et al (2003). Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. Gut, 52, 94-100.

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.

Silanes IL, Olmo N, Turnay J, et al (2004). Acquisition of resistance to butyrate enhances survival after stress and induces malignancy of human colon carcinoma cells. Cancer Res, 64, 4593.

Song K, Li M, Xu XJ, et al (2014). HIF-1α and GLUT1 gene expression is associated with chemoresistance of acute myeloid leukemia. Asian Pac J Cancer Prev, 15, 1823-9.

Tang L, Wei F, Wu Y, et al (2018). Role of metabolism in cancer cell radioresistance and radiosensitization methods. J Exp Clin Cancer Res, 37, doi: 10.1186/s13046-018-0758-7.

Tirino V, Camelrino R, Franco R, et al (2009). The role of CD133 in the identification and characterisation of tumour-initiating cells in non-small-cell lung cancer. Eur J Cardiothorac Surg, 36, 446-53.

van Staalduinen J, Baker D, Ten Dijke, P, van Dam H (2018). Epithelial-mesenchymal transition-inducing transcription factors: new targets for tackling chemoresistance in cancer?. Oncogene, 37, 6195-11.

Virag P, Fischer-Fodor E, Perde-Schrepler M, et al (2013). Oxalipatin induces different cellular and molecular chemoresistance patterns in colorectal cancer cell lines of identical origins. BMC Genomics, 14, 480.

Wang H, Qiu X, Lin S, et al (2018). Knockdown of IFI27 inhibits cell proliferation and invasion in oral squamous cell carcinoma. World J Surg Oncol, 16, doi: 10.1186/s12957-018-1371-0.

Wang T, Ning K, Lu TX, Hua D (2017). Elevated expression of Trp5C5 and GLUT1 is associated with chemoresistance in colorectal cancer. Oncol Rep, 37, 1059-65.

Yan D, Tu L, Yuan H, et al., (2017). WBSCR22 confers oxalipatin resistance in human colorectal cancer. Sci Rep, 7, 15443.

Zhan HX, Xu JW, Wang L, et al (2015). FoxQ1 is a novel molecular target for pancreatic cancer and is associated with poor prognosis. Curr Mol Med, 15, 469-77.

Zheng H-C (2017). The molecular mechanisms of chemoresistance in cancers. Oncotarget, 8, 59950-64.

Zhang H, Meng F, Liu G, et al (2011). Forkhead transcription factor foxq1 promotes epithelial-mesenchymal transition and breast cancer metastasis. Cancer Res, 71, 1292-301.

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.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.