Anti-cancer effects of butyrate: use of micro-array technology to investigate mechanisms

Elizabeth A. Williams*, Jonathan M. Coxhead and John C. Mathers
Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK

Epidemiological evidence suggests that a high intake of resistant starch and NSP protects against colo-rectal cancer. The mechanisms underlying this protection are thought to be mediated by the short-chain fatty acid butyrate, which is present in the colonic lumen in millimolar concentrations as a result of bacterial fermentation of carbohydrates that have resisted digestion in the small intestine. In vitro studies have shown that butyrate displays a host of chemo-preventative properties including increased apoptosis, reduced proliferation, down regulation of angiogenesis, enhanced immunosurveillance and anti-inflammatory effects in colo-rectal cancer cell lines. However, the molecular mechanisms underlying the apparent chemo-preventative actions of butyrate are largely unknown. The evidence supporting the role of butyrate as an anti-cancer agent is reviewed, with particular emphasis on those studies that have attempted to elucidate the mechanism of action of butyrate. Our understanding of the mechanistic action of butyrate and its role in cancer prevention is likely to advance considerably in this post-genomic era with the application of genomic and proteomic technologies. Studies are described that have used gene array and proteomic techniques to investigate the response of colo-rectal cancer cells to butyrate. These pioneering studies illustrate the potential of these technologies to help characterise the molecular responses of the cancer cell to butyrate, and to define the role of butyrate (and other nutrients) in the prevention of colo-rectal cancer.

Butyrate: Micro-array techniques: Colo-rectal cancer

Butyrate is an abundant anion in colonic contents where it occurs at concentrations of 2–10 mM (Cummings et al. 1987). As with the other major short-chain fatty acids (acetate and propionate), butyrate is an end product of bacterial fermentation of carbohydrates that have escaped digestion in the small bowel. Higher intakes of these structurally-disparate fermentable carbohydrates, which include resistant starches, NSP and oligosaccharides, are associated with reduced risk of colo-rectal cancer (CRC) (Bingham, 1990; World Cancer Research Fund and the American Institute of Cancer Research, 1997). The mechanisms underlying this putative chemo-preventative effect are poorly understood, but the strongest evidence is based on the anti-neoplastic actions of butyrate. Most of this evidence comes from in vitro studies, but D’Argenio et al. (1996) showed that direct administration of butyrate into the colon of rats treated with the carcinogen azoxymethane reduced the number and size of tumours. The present review summarises the evidence for the tumour-suppressing activity of butyrate (Fig. 1) and indicates the promise offered by transcriptomics (in the form of cDNA micro-array technology) and proteomics to help elucidate the mechanism of action of butyrate.

Suppression of cell proliferation

For at least two decades it has been well established that butyrate in low millimolar concentrations suppresses the growth of a range of animal cells (Prasad & Sinha, 1976; D’Anna et al. 1980; Kruh, 1982; Wintersberger et al. 1983), including CRC cell lines (Whitehead et al. 1986; Gamet et al. 1992; Siavoshian et al. 1997). Exposure to butyrate results in arrest in the G1 phase of the cell cycle and is associated with the induction of terminal differentiation (Leder & Leder, 1975; Siavoshian et al. 2000). Progression through the G1 phase of the cell cycle requires inactivation of the retinoblastoma protein, a phosphoprotein of 105–114 kDa located in the nucleus (Weinberg, 1995). This inactivation

Abbreviations: CRC, colo-rectal cancer; DAF, decay accelerating factor; IL, interleukin; TSA, Trichostatin A.
*Corresponding author: Dr Liz Williams, fax +44 191 222 8684, email e.a.williams@ncl.ac.uk
butyrate may be mediated.

Potential mechanisms by which the anti-neoplastic actions of butyrate and of the universal cyclin-dependent kinase inhibitor p21 have been interpreted as markers of induction of differentiation (Steinman et al. 1994; Kiers et al. 1995). Kruh et al. (1995) reviewed the evidence that differentiation induced by butyrate alters the morphology and ultrastructure of tumour cells, including changes to cell surface mucopolysaccharides and glycolipids, resulting in the loss of most of the malignant characteristics of the cancerous cell.

**Induction of apoptosis**

Resistance to death by apoptosis is one of the hallmarks of cancer cells (Hanahan & Weinberg, 2000) and this factor, together with maintained or enhanced rates of cell proliferation, contributes to expansion of the tumour mass. Hague et al. (1993) were the first to demonstrate that butyrate induced apoptosis in both adenoma and carcinoma cell lines, and reported that this induction of apoptosis was p53-independent (see also Mahyar-Roemer & Roemer, 2001). The latter is particularly interesting because p53 (often described as the ‘guardian of the genome’) is a transcription factor (Evan & Littlewood, 1998) that increases in concentration in response to DNA damage and results in growth arrest and repair, or in apoptosis of the damaged cell (Liu & Kulesz-Martin, 2001). At least 50 % of CRC tumours contain p53 mutations, rendering them susceptible to failure of apoptosis and increased accumulation of DNA damage. Heerdt et al. (1994) confirmed the ability of butyrate to induce apoptosis in colon tumour cells and proposed that this process occurred via a terminal differentiation pathway. Increased expression of the differentiation markers E-cadherin and alkaline phosphatase was observed in CRC cell lines treated with butyrate (Butt et al. 1997). More recent studies have begun to dissect the molecular mechanisms by which butyrate treatment results in tumour cell apoptosis, and have shown that butyrate up regulates expression of the pro-apoptotic protein BCL-2 homologous antagonist/killer (BAK) and induces caspase-3-mediated cleavage of target proteins, including poly-(ADP-ribose) polymerase (PARP) (Ruemmele et al. 1999; Clarke et al. 2001), the universal cyclin-dependent kinase inhibitor p21Waf1/Cip1 (Chai et al. 2000) and adenomatous polyposis coli (APC). The latter is cleaved after Asp737 to yield a characteristic and stable 90 kDa fragment (Browne et al. 1994, 1998; Webb et al. 1999).

**Anti-inflammatory actions**

Intestinal diseases such as ulcerative colitis and Crohn’s disease, which have an important inflammatory component in their pathology, carry an increased risk of CRC. Butyrate has anti-inflammatory effects (Inan et al. 2000; Saemann et al. 2000), which may be mediated by interference with the maturation of monocyte-derived dendritic cells (Saemann et al. 2002). Butyrate appears to shift the T helper-1–T helper-2 balance by inhibiting production of the pro-inflammatory cytokines interleukin (IL) 12, tumour necrosis factor-α and interferon-γ, whilst having no effect on, or stimulating, the release of IL-6 and IL-10 (Saemann et al. 2000, 2002; Nancey et al. 2002). The molecular mechanism for this shift is unclear, although there is evidence of suppression of nuclear factor kappaB activation in some (Inan et al. 2000) but not all (Diakos et al. 2002) studies. Faust et al. (2001) have argued that the anti-inflammatory actions of butyrate in the intestinal mucosa are unlikely to occur by direct regulation of cytokine-induced anti-inflammatory protein expression. Recent data from Diakos et al. (2002) indicate that at relatively low concentrations (1 mM) butyrate inhibits production of the growth factor IL-2 (which is required for expansion of antigen-specific T cells) by decreasing nuclear binding of the transcription factor nuclear factor of activated T cells in both Jurkat cells and in primary T cells.

**Up regulation of immunosurveillance**

Although still a controversial concept (for a review, see Armstrong & Mathers, 2000), it is becoming clearer that some chemo-preventative agents may act by up regulating immunosurveillance. The mechanisms for enhanced immunosurveillance could include better ‘visualisation’ of tumour cells as foreign by the immune system and/or attenuation of the ability of tumour cells to fight back against immune cells. Perrin et al. (1994) reported that the growth of PROb cancer cells, injected into the peritoneum of rats, was suppressed by concurrent treatment with butyrate and recombinant IL-2. The weak immunogenicity of PROb cells seemed to be enhanced by butyrate treatment making the tumour cells more susceptible to IL-2-activated natural killer cells. Butyrate may increase the effectiveness of other anti-cancer agents, as shown recently for actinomycin D in a mouse melanoma model (Giermasz et al. 2002). Butyrate treatment decreased expression of decay-accelerating factor (DAF) in three colon cancer cells lines (HT-29, Caco-2 and T-84; Andoh et al. 2002). DAF is expressed on the plasma membrane of CRC cells, forming a barrier to complement-
mediated clearance by the humoral immune response so that
down regulation of DAF by butyrate appears to improve
immunosurveillance (Andoh et al. 2002). Bonnotte et al.
(1998) demonstrated that exposure of several human and rat
CRC cell lines to butyrate in vitro increased their sensitivity
to Fas ligand-mediated apoptosis in the absence of a change
in expression of the Fas receptor on the surface of the target
cells.

**Down regulation of angiogenesis**

Expansion of solid tumours, including colon carcinomas,
requires the production of a new blood supply (neovascular-
isation). As prevention of neovascularisation will limit the
growth of tumours, angiogenesis has become a very
attractive target for anti-tumour agents. The cyclo-
oxynogenase-2 selective inhibitor celecoxib, which has been
shown to suppress the appearance of colonic polyps in indi-
viduals with familial adenomatous polyposis (Steinbach
et al. 2000), may act in part via inhibition of angiogenesis.
There is now evidence that butyrate may also have anti-
angiogenic effects. Chemokines and their receptors are
involved in angiogenesis, and Jordan et al. (1999) showed
that HT-29 cells expressed the chemokine receptor CXCR4.
However, within 3 h of treatment with 5 mM-butyrate
CXCR4 expression by HT-29 cells was inhibited
completely (Jordan et al. 1999). Treatment of the same cell
line with 2 mM-butyrate down regulated expression of two
angiogenesis-related proteins i.e. vascular endothelial
growth factor, the most potent angiogenic factor, and
hypoxia-inducible factor-1α (Pellizzaro et al. 2002).

**Functional genomics and proteomics**

Over recent years the sequencing of the whole genome of
different organisms, including man, has provided the foun-
dation for several powerful new biotechnologies offering
insight into: (a) the molecular characteristics of the cell; (b)
the regulation of cellular activity; (c) the response of the cell
to its environment; (d) cellular defects in disease states.
Genomics, transcriptomics and proteomics are all based on
the faithful transcription of DNA from the coding region of
the gene into a complementary single-stranded mRNA
molecule and subsequent translation into the protein (Fig. 2).
Application of these technologies is revolutionising the
biosciences, but it is in medical sciences where they are
likely to have their biggest impact (Mohr et al. 2002).
Functional genomics is being used to characterise the
molecular events occurring in disease processes (Kitahara
et al. 2001; Luo et al. 2001), to identify polymorphisms in
genes that may be associated with increased (or decreased)
risk of specific diseases (Hacia & Collins, 1999; Sapolsky
et al. 1999) and to search for biomarkers of disease (Van
Eyk, 2001; Jones et al. 2002). In addition, these technologies
offer exciting opportunities to explore and characterise the
mechanistic action of nutrients at a molecular level. The
following is a brief overview of the technologies themselves
and an indication of how their application has begun to be
used to help elucidate the mechanism(s) underlying the anti-
neoplastic action of butyrate.

**Genomics and transcriptomics**

DNA array technology allows the expression of thousands
of genes to be analysed simultaneously. This high-
throughput approach is facilitating rapid advances in
genomics and transcriptomics. The principle underlying the
array technologies is hybridisation between complementary
single-stranded nucleic acid sequences exploiting the
specific relationship between A and T, and G and C
(Southern et al. 1999). The latest micro-arrays consist of
densely-packed spots of single-strand cDNA or oligo-
nucleotides complementary to the sequence of interest
immobilized onto a substrate, e.g. glass or nitrocellulose.
RNA is extracted from cells, reverse-transcribed using
fluorescently-labelled nucleotides and hybridised to the
array. The array is laser-scanned and expression levels
determined from the intensity of the fluorescence at each
spot. The results are processed through a database and data
mining tools used for analysis (Duggan et al. 1999).
Such experiments generate massive amounts of expression data,
the interpretation of which is challenging and dependent
largely on the quality of genomic databases and of the
software tools available. Good experimental design and
careful attention to experimental protocols are critical to the
success of array experiments. For example, homogeneous
populations of cells may be difficult or impossible to extract
from heterogeneous biopsy material, so that interpretation of
the gene expression data may become equivocal. In
addition, without appropriate precautions, less-abundant
mRNA may fall below the limits of detection. Array experi-
ments are often complemented by more conventional
molecular approaches, e.g. Northern or Western blotting and
reverse transcription–polymerase chain reaction to confirm
the expression data and/or to investigate the kinetics of
expression. mRNA expression does not necessarily correlate
with protein expression (Anderson & Seilhamer, 1997), and
does not account for post-translational modification of
proteins, so that an integrated approach using trans-
criptomics and proteomics can provide a powerful insight
into cellular activity.

**Proteomics**

The term proteome was coined by Wasinger et al. (1995) to
describe the protein complement of the genome. Proteomics
is the study of the protein properties of the cell, encompassing protein expression, protein interactions and post-translational modifications (Blackstock & Weir, 1999). At the core of the technology is two-dimensional gel electrophoresis, which separates proteins according to molecular weight in one dimension and isoelectric charge in the other (O’Farrell, 1975). The use of large two-dimensional gels allows thousands of protein spots to be resolved in a single gel (Klose, 1999). Gels may be stained using Coomassie blue, silver staining or a fluorescent dye, allowing visualisation of proteins, image analysis and spot detection. The position of a protein spot on the gel may give clues to its identification, but unambiguous protein identification depends largely on MS. For identification a protein spot is selected from the two-dimensional gel, digested with an appropriate protease and its composite peptides identified according to their mass using MS. The pattern of peptide masses provides a unique protein fingerprint that can be used to search protein databases to identify the protein itself. Proteins not identified through this approach are subjected to more advanced MS methods, e.g. matrix-assisted laser desorption–ionisation time-of-flight (MALDI-TOF) allowing partial peptide sequencing. Peptides can then be characterised using nucleic acid sequence databases to search for their sequence tags. Major improvements in MS techniques have led to improved protein identification and detection of post-translational modification which, coupled with the development of protein and nucleic acid databases, have resulted in considerable advances in the field.

Exploring the anti-neoplastic properties of butyrate using functional genomics

The application of functional genomics to define the mechanisms underlying the neoplastic properties of butyrate has thus far been confined to homogeneous cell cultures. These pioneering studies will be reviewed.

The first in the field

The first published study that used micro-array technology to investigate the effect of butyrate on the transcriptome of colonocytes was that of Mariadason et al. (2000). The transcriptional response of SW620 colon carcinoma cell line to 5 mM-butyrate was compared with responses to three other compounds, i.e. Trichostatin A (TSA; an effective histone deacetylase inhibitor), sulindac (a non-steroidal anti-inflammatory drug) and curcumin (a dietary chemoprotective agent). Expression profiles of 8063 gene sequences were analysed using micro-array technology at six time points up to 48 h post-treatment, and only those genes whose expression, once modified, was sustained until the end of exposure to the agent were included in the analysis. The results demonstrate a complex cascade of reprogramming of SW620 colonic epithelial cells in response to butyrate over time. Genes were recruited rapidly in response to butyrate exposure, with some changes in gene expression observed within 30 min. Subsequent time points were characterised by a progressive expansion of the population of differentially-expressed genes that began to moderate after 16 h. Over the 48 h a staggering 589 genes were reprogrammed (256 up regulated, 333 down regulated) in the presence of butyrate. Almost as many (534) genes were differentially expressed on exposure to sulindac, with less extensive changes following treatment with TSA and curcumin.

Detailed interrogation of the data showed that, despite the similarities in the phenotypic response of SW620 cells to the butyrate and to the non-steroidal anti-inflammatory drug sulindac, the underlying genetic responses were profoundly different. Both butyrate and sulindac induce G0–G1 cell cycle arrest, trigger an apoptotic cascade and up regulate β-catenin-T cell factor. Of the more than 500 genes that were differentially expressed in response to each of the agents, 145 genes were mutually responsive to both butyrate and sulindac exposure (Fig. 3). However, only ninety-two of these genes responded in the same manner to both compounds, with thirty-six up regulated and fifty-six down regulated. The compounds had opposite actions on the remaining fifty-three genes. The genes responsive to butyrate exposure were most comparable with the gene profile evoked by TSA treatment. Butyrate, sulindac and TSA all induce G0–G1 cell cycle arrest, yet there was more similarity between the cell cycle genes altered in response to butyrate and TSA than in response to sulindac. The greatest differences in response compared with that to butyrate were seen for curcumin, which arrests cells in G2–M. The authors hypothesised that the activity of butyrate and TSA as histone deacetylase inhibitors could account for the similarity in gene profiles. Both butyrate and TSA inhibited histone deacetylase (observed as an altered acetylated histone H4:histone H1 value, determined by Western blotting), but the kinetics profile of histone hyperacetylation differed for the two agents. These differential kinetics allowed the identification of two clusters of genes (not differentially altered by either sulindac or curcumin) that were activated in tandem with histone acetylation, suggesting that the changes in these genes occur as a consequence of inhibition of histone deacetylase activity. Mariadason et al. (2000) conclude that, in the context of CRC chemo-prevention, agents such as butyrate that induce death of tumour cells by reprogramming them along a ‘natural’ maturation pathway may offer greater advantages in terms of safety than, for example sulindac, which also induces tumour cell apoptosis but which is associated with side-effects such as mucosal ulceration when used over long periods. In addition, the revelation by micro-array analysis that two agents may induce tumour cell apoptosis by different pathways suggests the possibility of synergy, and opens opportunities for use of combinations of chemo-preventative agents.
Choosing the most informative time point

Before deciding on the conditions for an array experiment, Iacomino et al. (2001) confirmed that 4 mM-butyrate suppressed cell growth and induced differentiation in the colonic adenocarcinoma cell line HT29. Butyrate-treated cells accumulated in the G0 phase of the cell cycle and there was increased expression of the differentiation marker alkaline phosphatase. Protein expression of the cyclin-dependent kinase inhibitor p21Waf1/Cip1 was reduced, whilst the oncogene c-myc was reduced at 48–72 h. Based on these findings Iacomino et al. (2001) chose to perform their array experiment at 72 h incubation with butyrate when cell cycle arrest and cell differentiation were maximal. They used an Atlas cDNA array to characterise and quantify the expression of 588 genes, including those involved in cell cycle regulation, signal transduction, apoptosis, DNA synthesis and repair, transcription and the stress response. Of these 588 genes, the HT-29 cells expressed 119, with sixty genes being differentially expressed (thirty-nine up regulated and twenty-one down regulated) in response to butyrate. In this study a twofold change in expression was considered significant. A cluster of genes regulating apoptosis, DNA synthesis, repair and recombination were among those up regulated, whilst a group of oncogenes, cell cycle regulators and transcription factors were down regulated. Reverse transcription–polymerase chain reaction was performed on a random selection of genes to confirm the array results, and on a sub-set of genes that were strongly responsive to butyrate.

Among the interesting findings of this study was the observation that butyrate treatment up regulated expression of a number of genes involved in the detoxification of xenobiotics. This finding suggests that increased butyrate supply to colonocytes might modulate the damage caused by carcinogens and/or alter the sensitivity to anti-tumour drugs. The response of some genes was unexpected, leading the authors to postulate on the role of these genes in the butyrate response. For example, they describe pro- and anti-apoptotic mRNA being concurrently up regulated, a feature that could be explained by deranged activity of cancer cells. In single-time-point micro-array studies such as this study, primary responses to the intervention cannot be distinguished from downstream events, and early events may be missed entirely if they occur transiently in advance of the chosen sampling time point. These possibilities complicate the interpretation of such data.

Focusing on mRNA expression to avoid confounding by downstream events

Della Ragione et al. (2001) combined aspects of the previous two studies to provide an elegant solution to the problem of downstream events masking the direct effect of butyrate on gene expression. These authors investigated the effects of treatment with butyrate or TSA in the presence of cycloheximide, to inhibit de novo protein synthesis, on the expression profile of HT29 cells. In this way they were able to observe transcriptional effects only. HT29 cells were treated with 2 mM-butyrate or 0.3 μM-TSA in the presence of cycloheximide for 5 h. Using the Atlas cDNA array of 588 genes, it was observed that the expression of twenty-three genes was modulated in an identical manner (two down regulated and the remainder up regulated) by both butyrate and TSA. Since TSA is a well-characterised histone deacetylase inhibitor, these data provide strong evidence that the initial responses (up to 5 h after exposure) to butyrate treatment occur via histone deacetylation inhibition.

Increased acetylation of histones would be expected to open up the chromatin structure and to increase transcription (Ballestar & Esteller, 2002), so the up regulation of expression of twenty-one genes accords with this hypothesis (Della Ragione et al. 2001). What is less clear is the extent to which there is selectivity in this response. In other words, does butyrate treatment lead to a generalised hyper-acetylation of DNA? The available evidence obtained using conventional gene expression methods (Krüh et al. 1995) and the more recent micro-array techniques (Mariadason et al. 2000; Della Ragione et al. 2001; Iacomino et al. 2001) suggest that exposure to butyrate up regulates expression of only a relatively small number of genes, but it should be remembered that the cDNA micro-array techniques are insensitive to small changes in expression (usually less than a twofold change).

In the study carried out by Della Ragione et al. (2001) only two of the differentially-expressed genes had been reported to be modulated by butyrate in previous studies. The remainder of the genes, whose functions included cell cycle regulation and DNA repair, may be potentially fruitful areas for further investigations. These authors used Northern blotting and reverse transcription–polymerase chain reaction to confirm mRNA data, and Western blotting to examine protein expression of two genes i.e. Tob–1 (an anti-proliferation mediator that acts synergistically with p21) and GATA–2 (a nuclear transcription factor). In addition, they provided direct evidence of the anti-proliferative properties of Tob–1 by transfecting HT29 cells with Tob–1 cDNA and observing a reduction in [3H]thymidine incorporation that correlated with the extent of Tob–1 protein expression.

Concordance between array experiments

Iacomino et al. (2001) and Della Ragione et al. (2001) both investigated the response of HT29 cells to butyrate exposure using the same ATLAS cDNA array. Of the twenty-three genes induced after 5 h exposure to 2 mM-butyrate in the presence of cycloheximide in the experiment of Della Ragione et al. (2001), eleven were also induced after 72 h exposure to 4 mM-butyrate in the study of Iacomino et al. (2001) (Fig. 4). Of the eleven genes differentially expressed 12

Study 1: 2 mM-butyrate + 20 μM-cycloheximide for 5 h
11
49
Study 2: 4 mM-butyrate for 72 h

Fig. 4. The number of genes differentially expressed by HT29 cells exposed to butyrate. Study 1, Della Ragione et al. (2001); study 2, Iacomino et al. (2001).
in both experiments nine were up regulated in both studies and two were up regulated after 5 h (Della Ragione et al. 2001) but down regulated after 72 h (Iacomino et al. 2001). Whilst aspects of the experimental conditions differed (butyrate concentration, time-frame and use of cycloheximide), the similarities in the gene profiles in response to butyrate (Table 1) offer potential targets for further study.

Several scenarios could explain the differences in gene expression in the two experiments including: (a) genes activated in the absence of cycloheximide may be downstream events; (b) there may be an overall expansion of the number of differentially-expressed genes between 5 and 72 h (Mariadason et al. 2000); (c) genes may be activated by butyrate in a concentration-dependent manner.

### Table 1. Genes in HT29 cells whose expression was modified by butyrate treatment

<table>
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<tr>
<th>Description</th>
<th>GenBank accession no.</th>
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<th>Direction of change</th>
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<td>Mapkap kinase</td>
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Table 1.

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↑. Genes up regulated; ↓. genes down regulated. *Study 1, genes differentially expressed in HT29 cells treated for 5 h with 2 mM-butyrate in the presence of 36 µM-cycloheximide (Della Ragione et al. 2001); study 2, genes differentially expressed in HT29 cells treated for 72 h with 4 mM-butyrate (Iacomino et al. 2001).

Applying the proteomics approach

Tan et al. (2002) used proteomics to help elucidate the mechanism of action of butyrate in colonocytes. Total cell lysates from HT29 cells treated with or without butyrate (5 mM for 24 h) were analysed by two-dimensional gel electrophoresis. The gel resolved over 1000 proteins, and comparison of the gels derived from butyrate-treated and control cells revealed regions of the gel showing differential protein expression patterns. MALDI-TOF MS was then used to identify thirty-five of the differentially-expressed spots. The proteins were found to be predominantly of cytosolic or mitochondrial origin. The authors reviewed the putative functions of many of the proteins differentially expressed in relation to butyrate and observed that, in addition to altered expression of proteins involved in the cell cycle, apoptosis and transcriptional regulation (the usual suspects), there was also altered expression of components of the ubiquitin–proteasome system. Tan et al. (2002) hypothesised that butyrate may play a role in regulation of these fundamental cell processes by altered proteolysis of ubiquitinated cellular proteins. For example, it is known that expression of p21Waf1/Cip1 (involved in butyrate-mediated G0–G1 arrest) shows proteosome-dependent regulation (Braggskomly et al. 1996).

Conclusions and future developments

These studies demonstrate how transcriptomic and proteomic experiments can help to elucidate the mechanisms underlying the chemo-preventative properties of butyrate. This work is in its infancy and, given the considerable differences in experimental protocol (e.g. cell line, dose of butyrate, time of exposure, level of expression (mRNA v. protein)) used by the different authors, it is unsurprising that there are marked differences in the genes identified as being differentially expressed. However, there is consistent evidence of suppression of CRC cell growth and enhancement of apoptosis, with growing support for the hypothesis that inhibition of histone deacetylation is the probable first step in the molecular action of butyrate.

Expression profiling at the mRNA and protein levels is now established and it offers a powerful route to increased understanding of mechanisms underlying the anti-neoplastic effects of butyrate and other food-derived agents. Rather than focusing on one or a small handful of specific genes or proteins (often chosen largely for pragmatic reasons), transcriptomic and proteomic approaches allow us to consider all the genes transcribed in a cell at a given instant. The challenges are to design protocols that maximise the interpretable information from such studies and to use these data to develop and test specific mechanistic hypotheses.
Acknowledgement

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References


