Butyrate: a simple gut microbiota metabolite in the modulation of epigenetic mechanism

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Butyric acid is a short-chain fatty acid (SCFA) produced after bacterial fermentation of fibre in the gut. It is naturally found in dairy milk and milk products such as butter, cheese, etc. Butter which consists of 3–4% butyric acid in the form of tributyrin (butyryl triglyceride), can be considered as a good dietary source of butyric acid. This fermented product besides being an energy source for intestinal epithelial cells can control different cellular processes in the body through epigenetic modification. Epigenetics involves modification of chromatin to regulate gene expression without altering the nucleotide sequence. It includes DNA methylation, histone proteins modification and chromatin remodelling which are associated with physiological and pathological processes. Butyrate is a well-known epigenetic nutrient as a histone deacetylase (HDAC) inhibitor, which acts as an anti-proliferative and differentiation agent by modulating the expression of cyclin D1 and $p21^{Waf1/Cip1}$ in normal mammary epithelial cells[1]. This anti-proliferative effect of butyrate makes it a chemo-preventive agent for the treatment of cancers. This SCFA is essential to maintain the normal intestinal health by bridging the communication between the gut and peripheral tissues. It can regulate the immune system of intestinal epithelial cells by regulating the modulating activity of different enzymes and transcriptional factors in the intestinal epithelial cells[2,3]. A butyrate analogue such as sodium butyrate induces the expression of antimicrobial peptide genes (TAP and β-defensin) to maintain the innate immunity of mammary epithelial cells[4]. Butyric acid analogues also have the property of inducing reprogramming of somatic cells to produce iPSCs which are mediated by *c-myc*[5]. Briefly, it indicates that butyrate is a potential therapeutic agent with clinical implications in human and veterinary medicine.

Epigenetics

In 1942, the term 'epigenetics' was coined by C. H. Waddington, which indicates functionally relevant changes to the genome that do not involve a change in the nucleotide sequence. In 2008, Cold Spring Harbor, USA gave a consensus definition of epigenetic trait as 'stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence'[6]. Chromatin is a highly dynamic structure that must be modified to allow the binding of transcriptional machinery when gene expression is required to allow for DNA replication or DNA repair mechanisms. Epigenetic mechanisms such as DNA methylation and histone modification regulate access of the transcription machinery to the target genes, modulating transitions from the condensed heterochromatin to the relaxed euchromatin, and vice versa[7]. Methylation usually occurs in and around gene promoters which are rich in cytosine and guanine nucleotides during post-transcription modification. These epigenetic mechanisms work synergistically to regulate the chromatin structure, and to achieve the required degree of gene expression required for normal physiological cell functions and a wide variety of biological processes. Due to the ability of the external and internal environmental factors to induce modifications in the genome, this epigenetic mechanism can be used to identify unknown etiology of many diseases. Presently, these epigenetic studies focus on embryonic development, ageing, cancer, inflammation, obesity, insulin resistance, intestinal bowel disease, type-2 diabetes mellitus, cardiovascular diseases, neurodegenerative diseases and immune diseases[8].

Colorectal cancer preventive and therapeutic agent

Colorectal cancer is the third and second most common cancer in men and women worldwide respectively[9]. The occurrence of colorectal cancer in humans is strongly influenced by environmental factors, including nutrients. Dietary fibres fermentation in the colon releases SCFA metabolites such as acetate, propionate and butyrate with highest concentration of 70–140 mM in proximal colon and lowest of 20–40 mM in distal colon with molar ratio of 60 : 25 : 15 respectively, depending upon diet, site of fermentation and microbiota composition[10]. Gut microbiota digest these non-digestible carbohydrates to produce SCFAs like acetate, propionate and butyrate. *Faecalibacterium prausnitzii* and *Eubacterium rectale* are the two most dominant species in gut microbiota (≈13–14% of the total faecal gut microbiota), and they contribute significantly to butyrate production[11]. Normal colonocytes use butyrate as a primary source of energy through mitochondrial β-oxidation, while colon cancer cells mainly rely on glucose as their primary energy source. This leads to accumulation of butyrate in the nucleus of cancerous cells, where it acts as a HDAC inhibitor. This is based on a mechanism known as 'Warburg effect'[12]. Cancer, enhances histone acetylation activity to upregulate different target genes, which in turn stimulate proliferation of normal colonocytes but inhibit proliferation of cancerous colonocytes due to inhibition of the Warburg effect. Modulation of the canonical Wnt signalling pathway is also one of the mechanisms by which butyrate protects the colon epithelial cells against colon cancer, which are constitutively activated in most colorectal cancers[13].

Gut microbiome metabolite in epigenetic modification of the immune system

Dendritic cells (DCs) are antigen presenting cells in the immune system that in addition to presenting antigens to the T-naive cells, also produce cytokines which induce the production of subsets of T-helper cells. Nastasi *et al.*[10] have hypothesized that bacterial metabolites such as butyrate in the gut exert immunomodulatory effect on human DCs after entering the tissues through blood circulation. These metabolites have been shown to modulate different signalling pathways in immune cells. They exert

their action through G-protein coupled receptors, which include GPR43 (FFAR2), GPR41 (FFAR3) and GPR109A (HCAR2). Nastasi *et al.*[10] showed that human monocyte-derived DCs have been acted upon by butyrate mainly through GPR41 and GPR109A. They observed that butyrate could significantly reduce LPS-induced IL6 mRNA expression and IL-12p40 protein, and increase *IL12B* gene expression in human monocyte-derived, mature DCs, but not in immature DCs. They also showed butyrate-specific chemokine production changes in mature DCs using LEGENDPlex™ assay; butyrate significantly decreased the secretion of chemokines such as CXCL9, CXCL10, CXCL11 and CCL4 by mature DCs. It indicates that butyrate has the ability to alter the inflammatory response in immune cells by regulating the levels of different cytokines, chemokines, interleukins, etc. Butyrate analogs such as sodium butyrate protect the immune system of bovine mammary epithelial cells from *Staphylococcus aureus* by inhibiting its internalization and by increasing the expression of antimicrobial peptides such as tracheal antimicrobial peptide and β-defensins in mammary epithelial cells. Butyrate highly induced the expression of TAP at two-fold level and β-defensin at three-fold level in *S. aureus*-inhibited mammary epithelial cells. Hence, butyrate could protect the mammary epithelial cells from microbial infections such as mastitis in bovines[4].

Neuro-protection under the control of gut microbiome

Butyrate can also improve brain health. Besides being an energy metabolite, it can act as a ligand for a subset of G protein coupled receptors. It is hypothesized that microbial metabolites such as butyrate have the ability to prevent neurodegeneration in the brain by altering gene expression. This may thus lead to possible treatments and even prevent neurological disorders[14–16]. Serotonin (5-hydroxytryptamine) is a highly ubiquitous neurotransmitter, synthesized from tryptophan that plays a critical role in the regulation of various physiological functions. Yano *et al.*[17] showed that after incubation of RIN14B enterochromaffin cells with butyrate, 5-HT (hydroxytryptamine) levels were elevated in the cells, which corresponds to increase in THP1 (tryptophan hydroxylase) expression from RIN14B cells. It suggested that butyrate induces transcription of the rate-limiting serotonin biosynthetic enzyme THP1 by directly acting on enterochromaffin cells, which regulates the synthesis of serotonin in the cells[17–19]. The blood–brain barrier (BBB) is an intact, highly selective semipermeable membrane barrier, which controls the passage and exchange of nutrients and molecules between the circulatory system and brain parenchyma, and also maintains homeostasis of the central nervous system. The microbial metabolites also have an impact on the basic physiology of BBB, such as permeability[18]. Braniste *et al.*[20] reported that lack of gut microbiota and their metabolites such as butyrate has increased permeability of BBB in adult mice than those with gut microbiota. They showed that the butyrate-treated mice by increasing the expression of tight junction proteins such as occludin but not claudin-5 have shown to decrease the permeability by strengthening the tight junction of the BBB. It has been shown that butyrate treatment or mono-colonization of *Clostridium tyrobutyricum* in germ-free mice increased the histone acetylation in brain lysates[21]. This indicates that butyrate acts as a neuroprotective agent after being metabolized by gut microbiota, and can be used to treat and prevent neurological disorders such as Autism and Parkinson's disease.

Epigenetic remodelling of stem cells

Butyrate stimulation is a simple tool which provides an efficient method for reprogramming of various human adult somatic cells into induced pluripotent stem cells. Mali *et al.*[16] reported the stem cells reprogramming efficiency of butyrate

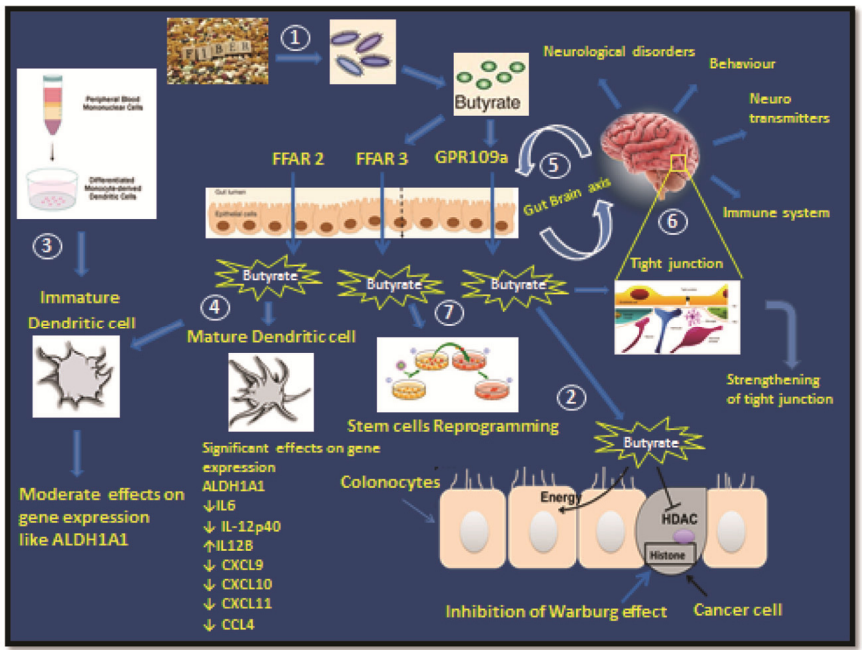

Figure 1. Schematic illustration of epigenetic effects of butyrate indifferent cells. (1) Butyrate produced after fibre digestion enters into blood circulation and crosses intestinal epithelial cells through G protein coupled receptors (FFAR2, FFAR3 and GPR109a). (2) Butyrate is partially used as energy source in colon cells, but inhibits the proliferation of cancerous epithelial cells through inhibition of Warburg effect. (3) Isolation of peripheral blood mononuclear cells (monocytes), culture of monocyte-derived dendritic cells (DCs), isolation and separation of immature and mature DCs. (4) Butyrate has moderate effecton expression of aldehyde dehydrogenase 1, but has no effects on other genes like chemokines and cytokines in immature DCs, while it significantly reduces the expression of IL6, IL-12p40, CXCL9, CXCL10, CXCL11, CCL4 and increases the expression of IL12B in human monocyte-derived mature DCs. (5) Butyrate improves brain health by regulating neurotransmitters, immune system and psychic behaviour and as a chemotherapeutic agent for neurological disorders. (6) Butyrate strengthens the tight junction of blood–brain barrier by increasing the expression of tight junction proteins such as occludin. (7) Butyrate efficiently converts partially reprogrammed cells into fully reprogrammed induced pluripotency stem cells.

using different adult somatic cells. They observed that butyrate analogue, viz. sodium butyrate was more stimulatory which elevated the reprogramming efficiency of human foetal IMR90 fibroblast by 51-fold compared to valproic acid, RG108 and BIX01294. Furthermore, using fluorescence activated cell sorting, they substantiated the stimulatory effect of butyrate through observation of TRA-1-60 expression at the single-cell level. They also reported the expression of pluripotency markers in butyrate-stimulated, established human iPSCs such as MR45 and MR46 (ref. 16). It indicated that butyrate efficiently elevates the reprogramming of stem cells and helps increase iPSCs with full differentiation functionality. However, the mechanism by which butyrate acts still is unknown. Researchers have attempted to explain the butyrate-mediated mechanism of elevation of reprogramming of stem cells using different approaches. Butyrate increases the histone acetylation activity but decreases the histone deacetylase activity. Butyrate stimulation also depends upon the reprogramming factors which are used in the reprogramming of cells. Hence, butyrate efficiently converts partially reprogrammed cells into fully reprogrammed, induced pluripotency stem cells.

Conclusion

Butyrate is a fibre digested metabolite having multi-functional role as a therapeutic for different organs such as brain, colon, etc. both in its pharmacologic and dietary form. Figure 1 summarizes the various epigenetic mechanisms through which butyrate influences the health of different cells and organs in different diseases and disorders. The dietary sources of butyrate such as butter, cheese and high-fibre diet potentially improve the health of the cells. As a chemotherapeutic agent, it is capable of targeting multiple pathways with different mechanisms of action which are disease-specific. However, the action of butyrate on germ cells remains unknown. Hence, more research is required to understand and establish the standard concentration and mechanisms of action of butyrate in different cells.

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