DNA mismatch repair status may influence anti-neoplastic effects of butyrate

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Abstract  
HNPCC (hereditary non-polyposis colon cancer) is an autosomal-dominant disorder characterized by early-onset CRC (colorectal cancer). HNPCC is most often associated with mutations in the MMR (mismatch repair) genes $hMLH1$, $hMSH2$, $hMSH6$ or $hPMS2$. The mutator phenotype of a defective MMR system is MSI (microsatellite instability), which also occurs in approx. 15–25% of sporadic CRC cases, where it is associated with the hypermethylation of the promoter region of $hMLH1$. Dietary factors, including excessive alcohol consumption, ingestion of red meat and low folate intake, may increase the risk of MSI high tumour development. In contrast, aspirin may suppress MSI in MMR-deficient CRC cell lines. Butyrate, a short-chain-fatty-acid end product of carbohydrate fermentation in the colon, shares a number of anti-neoplastic properties with aspirin, including inhibiting proliferation and inducing apoptosis of CRC cells. Recent in vitro studies suggest that physiological concentrations of butyrate (0.5–2 mM) may have more potent anti-neoplastic effects in CRC cell lines deficient in MMR, but mechanisms for such a differential response remain to be established.

Introduction  
Tumour cell development is a Darwinian process in which the tumour cell acquires a competitive advantage [1], such as enhanced proliferation, a survival advantage, in the form of apoptotic resistance, or increased metastatic potential. In the large bowel, tumours arise from the colonic epithelium as the result of a multi-step process. This can be seen histologically in the well known adenoma–carcinoma sequence, in which the histological changes are a consequence of genetic and epigenetic events resulting in loss of function (or silencing) of tumour-suppressor genes and activation of oncogenes.

HNPCC (hereditary non-polyposis colon cancer), MMR (mismatch repair) and MSI (microsatellite instability)  
Cells defective in DNA mismatch repair (MMR) generate mutations at a rate 1000-fold greater than observed in normal cells [2,3], which generates a Darwinian advantage. Individuals with a germline mutation in a MMR gene (usually $hMLH1$, $hMSH2$, $hMSH6$ or $hPMS2$) belong to HNPCC families, which have increased susceptibility to developing CRC (colorectal cancer) [4]. HNPCC is an autosomal-dominant disorder characterized by early onset CRC, with individuals presenting at an average age of 44 years compared with 65 years in sporadic CRC [5]. HNPCC accounts for approx. 2% of the total CRC burden when assessment is based on clinical criteria, with the $bMLH1$ gene being mutated most frequently (approx. 50% of the mutation spectra) among HNPCC families [6]. The mutator phenotype of HNPCC is MSI, characterized as either a shortening or a lengthening of the original DNA template caused by the incorrect replication of repeated DNA sequences [7]. MSI also manifests in approx. 15–25% of sporadic CRC cases [6] and is associated with hypermethylation of CpGs in the promoter region, and therefore silencing, of $hMLH1$ [8].

Aspirin and MMR  
Ruschoff et al. [9] were the first to show that the exposure of MMR-deficient HCT116 ($bMLH1^{-}$) cells to the non-steroidal anti-inflammatory drug aspirin could alter the mutator phenotype of a MMR-deficient cell line. HCT116 cells treated with aspirin showed a time- and dose-dependent reduction in MSI. Ruschoff et al. [9] hypothesized that aspirin exposure resulted in genetic selection for cells with microsatellite stability and that microsatellite-unstable cells were selected out by apoptosis.

Diet and MMR  
Up to one third of CRC can be attributed to dietary influences [10], but information on the influence of diet on the MMR system or MSI in the colon is limited. Epidemiological evidence suggests that an excessive consumption of alcohol, particularly as spirits, is a risk factor for developing colonic tumours showing MSI [11]. Ingestion of red meat has been...
associated with MSI tumours in the proximal colon, with an increase in incidence if the cooking methods have promoted the production of mutagenic HAs (heterocyclic amines) [12]. In vitro studies have shown a heightened mutation frequency of HCT116 CRC cells exposed to one of the HAs associated with grilled meat, PhIP (2-amino-1-methyl-6-phenyl-imidazo-[4,4-b]-pyridine). MMR corrected HCT116 + chr3 cells (HCT116 cells with bMLH1 restored by transfection with chromosome 3) exposed to PhIP showed fewer mutations and a reduction in cell viability [13]. In contrast, dietary antioxidants appear to be protective against oxidative damage in MMR-deficient cells. HCT116 cells exposed to ascorbate were reported to show a reduction in spontaneous mutation in MMR-deficient cells. HCT116 cells exposed to ascorbate showed greater inhibition of proliferation and higher rates of apoptosis following 1 mM butyrate exposure in the bMLH1-deficient cell line. Gene-expression analysis, by microarray of HCT116 and SW480 cells following 48 h of exposure to 1 mM butyrate, revealed that more genes were differentially expressed in the HCT116 cells, with many of the differentially expressed genes associated with proliferation, apoptosis and immunosurveillance [21]. MSI analysis of SW480 and HCT116 cells, following 20 days of 1 mM butyrate treatment, showed that butyrate appeared to elevate MSI in the HCT116 cell line, whereas there was no effect on the SW480 cells [23].

In summary, MMR-deficient CRC cells may be more susceptible to the anti-neoplastic effects of butyrate, but the mechanisms of this putative differential response remain to be established.

Butyrate and chemoprevention

Butyrate is a short-chain-fatty-acid end product of carbohydrate fermentation in the colon and is regarded as a key compound in the maintenance of a healthy mucosal lining, with colonocytes actively proliferating under its influence in normal tissue [16]. However, butyrate is also one of the most potent anti-neoplastic agents of the colon, associated with increasing apoptosis, inhibiting proliferation (and inducing differentiation), improving immunosurveillance, reducing angiogenesis and suppressing inflammation [17].

Butyrate and MMR

Many of the above-mentioned anti-neoplastic properties associated with butyrate are also well-documented for aspirin, and studies have suggested that a combination of the two compounds has an enhanced effect on inhibiting proliferation and inducing apoptosis of CRC cells [18,19]. Our previous studies have investigated the effects of butyrate on MMR-proficient and -deficient CRC cell lines [20–23]. An initial experiment investigated the effects of long-term (12 weeks) exposure to 1 mM butyrate on cell proliferation in MMR-deficient HCT15 (hMSH6−), HCT116 (bMLH1−) and LoVo (bMSH2−) CRC cells and MMR-proficient SW480 and HT29 CRC cells. Such butyrate exposure inhibited cell proliferation throughout the treatment period, and the effects were more pronounced in the cells that were defective for MMR. Normal rates of cell proliferation were restored when the cells were returned to normal (no butyrate) medium [20]. The MMR-deficient HCT116 cell line appeared to be particularly sensitive to the effects of butyrate, and further studies have shown that this bMLH1−defective cell line has a greater sensitivity to the effects of butyrate when compared with the MMR-proficient SW480 CRC cell line [21] and with the HCT116+chr3 cell line [22]. Both of these studies showed greater inhibition of proliferation and higher rates of

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References


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