Role of DNA-mismatch repair in anti-neoplastic effects of butyrate

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Colo-rectal cancer (CRC) is the second-most-common cause of cancer-related death in the Western world⁴. DNA-mismatch repair (MMR) genes regulate key cellular processes, including correction of DNA replication errors⁵. Impaired functioning of MMR has been implicated in the aetiology of hereditary non-polyposis colon cancer and in ≤15% of sporadic CRC⁶. The C₄ fatty acid, butyric acid, which is produced by bacterial fermentation of resistant starches in the large bowel, has potent anti-neoplastic effects on colon cancer cells⁷. Recent in vitro studies have indicated that MMR status may modulate the anti-neoplastic effects of butyrate⁸. The present study aimed to investigate the mechanisms underlying these differential effects of butyrate on colon cancer cells.

SW48 colon cancer cells, in which the MMR gene MLH1 is silenced by promoter hypermethylation, were treated with the demethylating agent 5-aza 2’deoxycytidine to partially demethylate and reactivate the MLH1 gene. The native SW48 cells and their demethylated counterparts were treated with butyrate (0–5 mM) for 8 d and the effects on cell proliferation, MLH1 gene promoter methylation (combined bisulfite restriction analysis assay) and expression of two butyrate-responsive genes, i.e. CDK4 and GADD45A, were assessed (real-time RT–PCR).

Butyrate (0.5–5 mM) suppressed proliferation (P<0.001) and reduced MLH1 promoter methylation (P<0.05) in SW48 cells. However, in demethylated SW48 cells butyrate caused a small but significant increase in cell proliferation (P<0.05; Fig. 1) and promoter methylation (P<0.05). CDK4 expression was higher (P=0.02) in demethylated SW48 cells compared with native SW48 cells. There was little effect of butyrate on CDK4 expression in SW48 cells, but this was reduced markedly in the demethylated cells (P=0.025 for cell line × butyrate interaction; Fig: 2). Further there was more than two fold up regulation of GADD45A expression following butyrate (1 mM) treatment in native SW48 cells as compared with demethylated SW48 cells in which GADD45A expression was down regulated (P=0.045 for cell line × butyrate interaction; Fig: 2).

The present study suggests that butyrate may have more potent anti-neoplastic effects on colon cancer cells, with epigenetic silencing of MLH1 function. Although butyrate showed differential expression of CDK4 and GADD45A genes, it does not explain its effects on cell proliferation. It is essential to investigate the effects of butyrate on more cell-cycle regulatory genes to understand the molecular mechanisms underlying these differential effects.

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