Untangling the Fiber Yarn: Butyrate Feeds Warburg to Suppress Colorectal Cancer

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Summary: Dietary composition has an important role in shaping the gut microbiota. In turn, changes in the diet directly impinge on bacterial metabolites present in the intestinal lumen. Whether such metabolites play a role in intestinal cancer has been a topic of hot debate. In this issue of Cancer Discovery, Donohoe and colleagues show that dietary fiber protects against colorectal carcinoma in a microbiota-dependent manner. Furthermore, fiber-derived butyrate acts as a histone deacetylase inhibitor, inhibiting cell proliferation and inducing apoptosis in colorectal cancer cells experiencing the Warburg effect. Cancer Discov; 4(12):1368–70. ©2014 AACR.

See related article by Donohoe and colleagues, p. 1387 (4).

Colorectal carcinoma is the third leading cause of cancer mortality in the world (1). This disease usually develops over many years via the accumulation of numerous genetic changes. Although some types of colorectal carcinoma are hereditary (2), most colorectal carcinoma cases are associated with diet and lifestyle (1). In line with this, the intestinal microbiota has been proposed to be a major contributor to the development of colorectal carcinoma (3). An increasing amount of data has demonstrated that dietary composition has an important effect on the gut microbiota, which, in turn, leads to changes in bacterial metabolites released to the intestinal lumen affecting intestinal tumorigenesis. In this context, dietary fiber is among the most-studied components of the diet in regard to the pathology of colorectal carcinoma. However, the role of fiber on colorectal carcinoma is controversial, mainly due to the fact that human cohort-based epidemiologic studies have yielded conflicting results. Furthermore, from those studies claiming a protective role, it is still unclear how fiber protects against colorectal carcinoma. Two possible mechanisms have been proposed. First, insoluble fiber may speed colonic transit, decreasing the exposure time of the colonic epithelium to carcinogens, and, second, intestinal bacteria can metabolize soluble fiber into metabolites with protective action, such as short-chain fatty acids (SCFA). In this issue, Donohoe and colleagues shed light on these controversies and elegantly demonstrate that, indeed, dietary fiber protects against colorectal carcinoma by increasing bacterial butyrate levels in the colon, which act as an histone deacetylase (HDAC) inhibitor, halting proliferation and promoting apoptosis of colon cancer cells (Fig. 1; ref. 4).

Genetic heterogeneity, differences in the composition of the gut microbiota, and the utilization of different sources of fiber are among the possible causes underlying the inconclusive results obtained from human studies (5). To overcome these hurdles, Donohoe and colleagues used BALB/c mice with a strictly defined gut microbiota kept on gnotobiotic isolators, thus avoiding colonization by other commensal bacteria (4). They then colonized some of the animals with Butyrivibrio fibrisolvens, a butyrate-producing bacterium, and fed the mice either low-fiber or high-fiber diets that were otherwise identical in composition and calorically matched. This experimental system allowed the authors to rule out any effect of differences in genetics, intestinal microbiota, and fiber source on colorectal carcinoma development. Using this gnotobiotic mouse model, they found that mice fed a high-fiber diet and colonized with B. fibrisolvens were protected against azoxymethane/dextran sodium sulfate (AOM/DSS)-induced colorectal carcinoma. Strikingly, these mice developed fewer, smaller, and less aggressive tumors than all the other experimental groups. Importantly, high-fiber diet per se did not have any protective effect on this colorectal carcinoma model, indicating that only in combination with the right microbiota could dietary fiber be beneficial in protecting against colorectal carcinoma. On the basis of these results, the authors propose that human epidemiologic studies should be revisited to incorporate differences in participants’ gut microbiota to better address the role of dietary fiber on colorectal carcinoma.

Another important conclusion one can immediately draw from this result is that a metabolic product from fiber fermentation by B. fibrisolvens must be involved in the tumor-suppressive effect of dietary fiber. In line with this possibility, mice fed a high-fiber diet and colonized with B. fibrisolvens had increased luminal levels of butyrate, but not acetate and propionate, the other two major SCFAs. This result clearly points to butyrate as a key bacterial metabolite inhibiting colorectal carcinoma development. To confirm this hypothesis, the authors modulated luminal butyrate levels by two different means. First, they colonized mice with a mutant B. fibrisolvens strain (that produces 7-fold less butyrate when cultured) and fed them a...
low-fiber or high-fiber diet as before. After AOM/DSS treatment, they found that mutant *B. fibrisolvens* conferred an attenuated protective effect to high-fiber diet in these mice. Alternatively, they provided control mice a tributyrin-fortified diet, which increases colonic butyrate levels independently of microbiota. Following the AOM/DSS regimen, these mice were almost completely protected against colorectal carcinoma, indicating that exogenous butyrate could recapitulate the protective effect of high-fiber diet and *B. fibrisolvens*. Together, these two experiments clearly demonstrated that fiber fermentation by *B. fibrisolvens* protects from colorectal carcinoma by increasing luminal levels of bacterial butyrate.

The tumor-protective effect of butyrate has been mainly attributed to its anti-inflammatory properties. Butyrate down-regulates the expression of proinflammatory cytokines in colonic macrophages, and it has been shown to regulate colonic regulatory T cells in mice, which have a crucial role in controlling intestinal inflammation (3). However, the authors did not find any difference in the number of regulatory T cells and associated cytokines among all the experimental groups, ruling out reduced inflammation as a cause for the protective effect of butyrate. On the basis of their previous work, the authors hypothesized that the tumor-suppressive role of butyrate in colorectal carcinoma could be related to the metabolic differences exhibited by normal and cancerous colonocytes. Butyrate represents the primary source of energy in normal colonic epithelial cells (6). However, colorectal carcinoma cells, like most cancer cells, display an increased glucose uptake and metabolism, a phenomenon termed the “Warburg effect” for the German scientist who originally described it in the early 20th century. Such a switch toward glycolytic metabolism is required to sustain their energetic and anaplerotic demands. As a consequence, butyrate is nontabolized in these cells to the same extent and, therefore, accumulates to such a concentration that it can act as an HDAC inhibitor (7). Indeed, the authors found increased levels of butyrate in the tumors of mice colonized with *B. fibrisolvens* and fed a high-fiber diet, suggesting that more butyrate molecules could be available to function as an HDAC inhibitor. Consistent with this, H3 acetylation levels are increased in the tumors of these mice, compared with adjacent normal colonocytes and tumors from control mice. Importantly, the authors found increased histone H3 acetylation at the promoter region of key proapoptotic and cell-cycle genes, such as *FAS*, *p21*, and *p27*, leading to increased expression of these genes and the concomitant inhibition of cell proliferation and induction of apoptosis of colorectal carcinoma cells. Finally, the authors extended these observations to human colorectal carcinoma samples, where they detected elevated levels of butyrate and H3 acetylation compared with matched normal mucosa.

Collectively, this body of work provides convincing evidence that dietary fiber, when combined with butyrate-producing bacteria, can protect from colorectal carcinoma by providing tumors with high levels of butyrate to act as an HDAC inhibitor, thus impairing tumor growth (Fig. 1). However, it also raises several intriguing questions. Which other species of bacteria are important in colorectal carcinoma protection? Although this study has focused on *B. fibrisolvens*, a type of bacteria common in ruminant animals, a large number of genera, including SCFA-producing species, have been identified in the human colon (8). In this context, different species could generate luminal butyrate at lower concentrations, inducing aberrant proliferation and transformation of colon epithelial cells, as recently reported in an *APC*mut*+/MSH*/ model of colorectal carcinoma (9). In the same way, can other bacterial metabolites play a role in
the tumor-suppressive effect of dietary fiber? The modest decrease in tumor protection shown in mice colonized with mutant *B. fibrisolvens* suggests that this very likely could be the case. It would be fascinating to elucidate which metabolites these are, and their effect on colorectal carcinoma prevention as well as in the metabolism of colon cancer cells. Finally, better knowledge of how to modulate our intestinal flora by changing our diet would definitely help us to elucidate the complex interaction between diet, intestinal flora, and colorectal carcinoma prevention.

**Disclosure of Potential Conflicts of Interest**

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