

## Agaro-oligosaccharides: a new frontier in the fight against colon cancer?

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THE HUMAN GUT REPRESENTS ONE of the most diverse bacterial ecosystems. These bacteria live in a mutualistic state with the host; however, when this homeostatic balance is perturbed by an alteration in bacterial community composition and function (dysbiosis), it can lead to disruption of normal host physiology. A role for gut microbiota has been suggested in pathogenesis of colorectal cancer based on recent mouse and human studies. In a mouse model of colitis-associated colorectal cancer, Uronis et al. (24) found that risk for tumorigenesis was lower in germ-free conditions than with a conventional microbiome. In another study Kostic et al. (11) found that *Fusobacterium* species were enriched in colorectal cancer patients. They attributed this enrichment to the strong adhesive and invasive abilities of *Fusobacterium* for epithelial cells, which allows them to take up residence in tumor environment. Kostic et al. also showed that introduction of *Fusobacterium* species in the *Apc<sup>Min/+</sup>* mouse model, a genetic mouse model of colon cancer, accelerates colonic tumorigenesis (11). These studies support a role for gut microbiota in the pathogenesis of colorectal cancer, but there is little known about factors that promote dysbiosis leading to colorectal cancer and how preventing dysbiosis might be protective. The present study by Higashimura et al. (10a) helps answer this question by investigating effects of high-fat diet (HFD) on gut microbiota and host function and supplementing HFD with a prebiotic to prevent changes in gut microbiota.

An important determinant of gut microbiota composition and function is diet. HFD consumption in particular is associated with alterations in gut microbiota and can lead to diseases including obesity and colon cancer (20, 23). Alterations in gut microbiota seen with HFD consumption that might be associated with colon cancer include decrease in *Bifidobacteria* and *Lactobacillus* spp. and increase in Firmicutes such as *Clostridium* and *Enterococcus* spp. (5). Although HFD-induced alteration in gut microbiota are associated with colon cancer, it remains unclear how these alterations promote colon cancer and whether prevention of these changes can halt progression to colon cancer.

There are several ways to target gut microbiota, one of which is a prebiotic, defined as “a non-digestible compound that through its metabolism by microorganisms in the gut, modulates composition and/or activity of the gut microbiota thus conferring beneficial physiological effects to the host” (4). Agarose, a major food ingredient in East Asia obtained from seaweed, can be considered a prebiotic given its effect on gut microbiota. In the present study Higashimura et al. (10a) utilized agarose-derived oligosaccharide (agaro-oligosaccharide; AGO) to determine whether AGO protects against HFD

induced gut dysbiosis and colon tumorigenesis. The authors show that HFD-fed mice over 8 wk have a significant increase in body weight as well as alterations in gut microbiota characterized specifically by a decrease in taxa within *Lactobacillales*, and increase within *Bacteroides* and *Clostridium* subcluster XIVa as measured by terminal restriction fragment length polymorphism analysis. These findings are similar to previous studies on the effect of HFD on gut microbiota and consistent with negative effects on the host (6, 21, 23). Enterotoxigenic *Bacteroides* have been implicated in tumorigenesis via activation of T helper cell 17 (T<sub>H</sub>17) (25). *Lactobacilli*, a member of *Lactobacillales*, on the other hand have previously been shown to be protective against proliferation of pathogenic bacteria (14, 17). AGO supplementation did not have an effect on weight gain but significantly suppressed the HFD-related changes in *Lactobacillales* and *Clostridium* subcluster XIVa. Although microbial compositional changes seen with AGO support its protective effects, the physiological effects on the host and changes in microbial community function in response to AGO are more relevant.

To determine whether AGO protects against colon tumorigenesis, the authors analyzed the effects of AGO supplementation on azoxymethane (AOM)-induced development of aberrant crypt foci (ACF) in HFD-fed mice. They found that AGO supplementation significantly suppressed the development of ACF in AOM-administered HFD mice. Furthermore, AGO also suppressed mRNA expression of tumorigenic factor cyclooxygenase-1 (COX-1) and increased anti-tumorigenic factor 8-oxoguanine DNA-glycosylase 1 (OGG1) in HFD-fed mice.

The authors in the present study also correlated changes in microbial composition to changes in microbial function to identify potential mechanisms by which AGO is protective. They found that lactic acid concentration was elevated in the cecum of HFD-fed mice that were supplemented with AGO, consistent with an increase in *Lactobacillales*. Succinic acid, a major by-product of *Bacteroides* metabolism, was also increased following HFD but suppressed by AGO supplementation. It is unclear whether these fermentative products directly influence colon tumorigenesis or are just reflective of changes in community function as a result of AGO supplementation.

In the present study, AGO supplementation prevented the decrease in cytoprotective  $\omega$ -muricholic acid (MCA) and increased the ratio of MCA to deoxycholic acid (DCA), providing a potential mechanism for anti-tumorigenic effect of AGO. A high physiological level of a secondary bile acid, particularly DCA, as seen with HFD consumption (15), has been reported to be a risk factor for colon cancer in humans (2, 3). DCA increases tyrosine phosphorylation of  $\beta$ -catenin, a member of the cadherin family of transmembrane cell-cell adhesion receptors and a key component of adherens junctions, which leads to enhanced colon cancer cell proliferation and invasiveness (13).

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A recent study has also shown that DCA levels are significantly higher in the sera of patients with colorectal adenomas (1), suggesting a link between DCA and colon tumorigenesis (16, 19). Since *Clostridium* spp. harbor a gene for 7 alpha dehydroxylation enzymatic activity (7, 18), decreased *Clostridium* spp. levels seen with AGO supplementation may in part be responsible for diminished DCA levels.

Apart from the prebiotic effects of AGO on microbial composition and bile acid production, AGO-mediated anti-tumorigenic activity might also occur via modulation of heme-oxygenase-1 expression (HO-1) and polyamine (PA) production. Recent studies have shown that AGO increases HO-1 expression in the macrophages (8, 10). Because increased HO-1 expression suppresses T<sub>H</sub>17 immune responses, inflammatory cytokines, and tumorigenic factors including TNF- $\alpha$  and COX-1 in mice (8, 26), it is possible that anti-tumorigenic effects of AGO and other oligosaccharides are in part HO-1 mediated. Evidence supporting this hypothesis is observed in the present study, in which COX-1 expression is suppressed by AGO in HFD-fed mice. Besides HO-1, AGO might have anti-tumorigenic effects by potentially reducing PA production in the intestine. PAs are a regulator of epithelial cell division and high PA levels have been linked to tumorigenesis (9). PAs in the human intestine can be produced by microbial species belonging to the *Clostridium* subcluster XIVa, which are suppressed by AGO (12, 22).

The present paper helps advance our understanding on beneficial physiological effects of prebiotics on the host in regards to colon cancer. The authors provide data to show that AGO, a prebiotic, suppresses HFD-induced dysbiosis, change microbial community function, and prevent colonic tumorigenesis in a HFD mouse model. Future studies investigating molecular mechanisms by which a prebiotic induces microbiota changes exerting protective effects on the host will allow development of new microbiota-targeted therapies.

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#### DISCLOSURES

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#### AUTHOR CONTRIBUTIONS

Y.B. drafted manuscript; Y.B. and P.C.K. edited and revised manuscript.

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