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A food-based approach that targets interleukin-6, a key regulator of chronic intestinal inflammation and colon carcinogenesis

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Abstract

Studies have shown a causal link between high-calorie diet (HCD) and colon cancer. However, molecular mechanisms are not fully elucidated. To understand etiology of HCD-induced colon carcinogenesis, we screened 10 pathways linked to elevated colonic cell proliferation and chronic inflammation in an HCD-consuming human-relevant pig model. We observed elevated colonic mucosal interleukin-6 (IL-6) expression in HCD-consuming pigs compared to standard diet controls (SD, $P=.04$), and IL-6 strongly correlated with Ki-67 proliferative index and zone, early biomarkers of colon cancer risk ($r=0.604$ and 0.743 and $P=.017$ and .002, respectively). Liquid chromatography–tandem mass spectrometry-based proteomic analysis and Ingenuity Pathway Analysis showed that HCD consumption altered IL-6 signaling pathway proteins (PI3KR4, IL-1α, Mapk10, Akt3, PIK3CG, PIK3R5, Map2k2). Furthermore, these proteins also correlated with Ki-67 proliferative index/zone. Anti-IL-6 therapeutics are available for treating colon cancer; however, they are expensive and induce negative side effects. Thus, whole foods could be a better way to combat low-grade chronic colonic inflammation and colon cancer. Whole plant foods have been shown to decrease chronic diseases due to the potential of anti-inflammatory dietary compounds acting synergistically. We observed that supplementation of HCD with anthocyanin-containing purple-fleshed potatoes (10% w/w), even after baking, suppressed HCD-induced IL-6 expression ($P=.03$) and the IL-6-related proteins IL-1α and Map2k1 ($P\leq.1$). Our results highlight the importance of IL-6 signaling in diet-linked induction/prevention of colonic inflammation/cancer and demonstrate the potential of a food-based approach to target IL-6 signaling.

Keywords: Colonic inflammation; Colon cancer; Interleukin-6; High-calorie diet; Purple-fleshed potatoes; Pig model

1. Introduction

Alongside the development of technology came a shift in our diet [1]. Westernized populations moved from hunter–gatherer lifestyles with minimal food processing to novel foods such as dairy products, cereals, refined sugars and fatty meats [1], common staples of the current Western diet. Furthermore, foods became easily accessible at low cost and increased convenience. Marked by high fat and high sugar, the Western diet is leading to a greater caloric intake in the society. This high-calorie diet (HCD) coupled with a sedentary lifestyle is linked to multiple diseases, including obesity, type 2 diabetes, cardiovascular disease and certain cancers such as colon cancer [2,3].

Colon cancer is the second leading cause of cancer-related deaths in the United States. Approximately 5% (1 in 20) of Americans will be diagnosed with colon cancer in their lifetime [4]. Consumption of an HCD has shown to increase the risk for colon cancer in a human model [5–7]. Recent animal studies also suggest a causal link between HCD and increased colon cancer risk [8–13]. Eighteen months of consumption of the Western diet induced colonic tumors in normal C57Bl/6 mice [13] in the absence of any carcinogen. Furthermore, a recent study by Erdelyi et al. [14] showed that the high-fat Western diet negatively impacted colonic lipid metabolism, oxidative stress, and immune responses in C57Bl/6 mice. Colonic inflammation plays an important role in elevating the risk for colon cancer with the implication of multiple pathways, including the interleukin-6 (IL-6) signaling pathway [15–17]. The association of IL-6 signaling with colon cancer was clearly demonstrated in a recent study by Day et al. [10]. These researchers found elevated IL-6

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expression in colonic polyps of $Apc^{Min/+}$ mice fed an HCD compared to those fed a standard diet (SD), suggesting that HCD can drive the increased production of proinflammatory cytokines, such as IL-6, thus elevating the risk for colon cancer.

IL-6 is a proinflammatory cytokine released by myeloid cells in various tissues crucial for immune response, cell survival, apoptosis and proliferation [18–21]. IL-6 also regulates the proliferation of intestinal epithelial cells [22]. Recent studies propose a link between chronic inflammatory diseases (*e.g.*, irritable bowel syndrome and colon cancer) and IL-6 signaling [16,18,20,23–25]. A study in C57BL/6 mice found higher IL-6 mRNA and protein expression in the dextran sodium sulfate and azoxymethane (DSS/AOM)-induced colon cancer tumors than surrounding normal colon tissue, suggesting that IL-6 may be responsible for enhanced colon carcinogenesis [26]. IL-6, when bound to its receptor IL-6R, leads to downstream activation of the JAK/STAT3 pathway, inducing expression of genes important in elevation of proliferation and suppression of apoptosis [18]. Recent work by Grivennikov et al. [18] in IL-6 knockout mice reported a decrease in Ki-67-expressing colon crypt cells, an early biomarker for colon cancer. Other studies have added evidence to propose that IL-6 acts in colon cancer by increasing proliferation (reviewed in [27]). Taken together, these studies suggest that IL-6 provides resistance to apoptosis and provides a conducive environment for increased cell proliferation, ultimately leading to enhanced cell growth and survival. Augmented IL-6 and its downstream signaling pathways may provide a proinflammatory milieu favorable for colon cancer development.

Currently, various anti-IL-6 therapeutics are being used or are in clinical trials for multiple diseases and cancers including colon cancer, multiple myeloma, prostate cancer, and Castleman disease [17,28]. The therapeutics available for colon cancer treatment currently target inhibition of the IL-6/STAT3 signaling pathway with anti-IL-6 receptor antibodies, soluble gp130Fc and small molecule JAK inhibitors [17]. The use of anti-IL-6 receptor antibodies has been shown to suppress the growth of colon tumors and protect against colon carcinogenesis in DSS/AOM-induced colon carcinogenesis in C57BL/6 mice [26]. While these therapeutics provide a potential therapy for cancer patients, therapeutics can be costly and include an array of negative side effects or even lead to drug tolerance [17]. Therefore, there is a growing interest in alternative therapeutics that could alleviate the cost and side effects patients face, reducing the stress of the already existing physical and psychological burden of disease.

Bioactive compounds from plants, including anthocyanins and phenolic acids, are linked to a reduced risk for a variety of cancers, including colon cancer [29–39]. However, individual phytochemicals have been shown to have proinflammatory and procancerous effects in high doses alone (reviewed in [40]). Plant foods have been illustrated in epidemiological studies and other research to hold a potential for disease prevention as different dietary ingredients can work synergistically to enhance the activity of a single compound, providing a better explanation for the benefits of whole foods observed in epidemiological studies [41–43].

Color-fleshed potatoes contain a variety of secondary metabolites, including polyphenols. Specifically, purple-fleshed potatoes are rich in phenolic acids and anthocyanins. Our previous studies linked the anti-colon-cancer properties of color-fleshed potatoes to their bioactive compounds [44–46]. Consumption of color-fleshed potatoes has been on the rise in the past 10 years, likely due to their putative health benefits. While studies on individual compounds and their anticancer effects have been performed, the benefits of plant foods such as anthocyanin-containing purple-fleshed potatoes, rich in anthocyanins, against colonic inflammation/cancer in humans or in a human-relevant model are lacking.

Developing an appropriate model for *in vivo* studies is crucial to best understand and extrapolate the data to human models. While mice are popular models for studying a variety of diseases, the anatomical and physiological differences between rodents and humans are significant. In addition, wide differences in dietary patterns exist between the two groups. The human is an omnivore, whereas rats and mice were originally granivores. Other models exist, including cats/dogs, but these models have differing diet and meal patterns than humans. Primates are rarely used in food intake studies due to their expense and scarcity [47]. In contrast, the pig is an excellent model to study the nutrition and food intake in humans. This study used a pig model because it is experimentally tractable and it is a clinically relevant model of the human gastrointestinal tract [47–49].

Using a human-relevant porcine model, we investigated the effect of HCD on colonic inflammation. We screened a panel of inflammatory biomarkers involved in colonic inflammation/cancer and identified the IL-6 signaling as the prominently altered pathway using quantitative polymerase chain reaction (qPCR) and proteomics analysis. Proteins in the IL-6 signaling pathway as well as IL-6 significantly correlated with Ki-67 proliferative index and zone, early biomarkers of colon cancer. Thus, our data revealed the role of IL-6 and its signaling pathway in enhancing colonic proliferation in colon cancer development in a human-relevant model. We further evaluated if dietary intervention of purple-fleshed potatoes could alleviate HCD-induced colonic inflammation. We witnessed suppression in IL-6 expression with the supplementation of only 10% w/w purple-fleshed potatoes, even after baking, in HCD-consuming pigs. Our study suggests staple crops that contain anthocyanins should be further as potential dietary interventions in the prevention and treatment of gastrointestinal inflammation/cancers.

2. Experimental section

2.1. Diet-induced inflammation

Male pigs (6 weeks old) were obtained from Smithfield Premium Genetics (Rose Hill, NC, USA) and housed individually in indoor pens at the North Carolina State University Swine Educational Unit (Clayton, NC, USA). The animals were allocated into different treatment groups by body weight so that mean initial body weight was similar among the treatment groups (N=8 animals/treatment).

2.2. Experimental diets

Animals were provided with one of the following diets: a SD (~5% fat), an HCD (17% added dry fat and ~3%–5% endogenous fat), or HCD supplemented with 10% of purple-fleshed potato (raw or baked). Purple-fleshed potatoes (Purple Majesty) were grown at Black Gold Farms (Pearsall, TX, USA). Baking of potatoes was done at Worldwide Food (Burley, ID, USA). Raw and baked potatoes were freeze-dried at Vandrunen Farms, IL, USA, prior to incorporation in the diet. White corn and dry fat were used as a major energy source, and soybean meal was the major protein source. Ratios between corn and soybean meal were adjusted to match energy and protein contents among diets. White corn was used to prevent carotenoids from yellow corn affecting the study. Composition of all the diets is presented in Table A1. Pigs consumed the experimental diets for 13 weeks — the feed and drinking water were provided *ad libitum*.

2.3. Colon tissue collection

The animals were euthanized at the end of the study using a captive bolt followed by exsanguination. The distal colon was resected and was cleaned with RNAse-free phosphate-buffered saline, and the mucosa was scraped using a glass slide into an RNAse-free tube. The tube was snap frozen in liquid nitrogen and later transferred to −80°C.