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Interactions between the colonic transcriptome, metabolome, and microbiome in mouse models of obesity-induced intestinal cancer

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Pfalzer AC, Kamanu FK, Parnell LD, Tai AK, Liu Z, Mason JB, Crott JW. Interactions between the colonic transcriptome, metabolome, and microbiome in mouse models of obesity-induced intestinal cancer. Physiol Genomics 48: 545–553, 2016. First published June 17, 2016; doi:10.1152/physiolgenomics.00034.2016.—Obesity is a significant risk factor for colorectal cancer (CRC); however, the relative contribution of high-fat (HF) consumption and excess adiposity remains unclear. It is becoming apparent that obesity perturbs both the intestinal microbiome and metabolome, and each has the potential to induce protumorigenic changes in the epithelial transcriptome. The physiological consequences and the degree to which these different biologic systems interact remain poorly defined. To understand the mechanisms by which obesity drives colonic tumorigenesis, we profiled the colonic epithelial transcriptome of HF-fed and genetically obese (DbDb) mice with a genetic predisposition to intestinal tumorigenesis (Apc1638N); 266 and 584 genes were differentially expressed in the colonic mucosa of HF and DbDb mice, respectively. These genes mapped to pathways involved in immune function, and cellular proliferation and cancer. Furthermore, Akt was central within the networks of interacting genes identified in both gene sets. Regression analyses of coexpressed genes with the abundance of bacterial taxa identified three taxa, previously correlated with tumor burden, to be significantly correlated with a gene module enriched for Akt-related genes. Similarly, regression of coexpressed genes with metabolites found that adenosine, which was negatively associated with inflammatory markers and tumor burden, was also correlated with a gene module enriched with Akt regulators. Our findings provide evidence that HF consumption and excess adiposity result in changes in the colonic transcriptome that, although distinct, both appear to converge on Akt signaling. Such changes could be mediated by alterations in the colonic microbiome and metabolome.

COLORECTAL CANCER (CRC) is the third most common cancer and third most common cause of cancer deaths in the United States, affecting approximately 137,000 additional people annually (35). Among the many risk factors for this disease is obesity: those with a body mass index (BMI) of 25–29.9 have a relative risk of 1.2 and 1.5 for developing CRC, while those with a BMI > 30 have a relative risk of 1.5 and 2.0 for females and males, respectively (23). Mouse studies corroborate the epidemiological findings and prove causality: both high-fat-induced obesity and genetically promoted obesity having been shown to significantly elevate tumor burden (5). Among gastrointestinal cancers, CRC is a good candidate for preventive strategies as there is a prolonged phase of development, estimated at 10 yr, during which normal mucosa evolves into a carcinoma (39). As the worldwide incidence of obesity remains stable, it is clear that strategies aimed at reducing the risk of CRC in the obese will require more than lifestyle and diet alterations.

Currently there are three prevailing hypotheses for the mechanism underlying the link between obesity and CRC where the central players are insulin resistance (34), adipokines (16, 42), and chronic inflammation (27). The first theory postulates that excessive insulin binding to the cell-surface insulin receptor leads to overactivation of downstream signaling events such as activation of insulin-receptor substrate proteins and the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway (34). The second theory relates to the elevation in leptin and suppression of adiponectin in obese individuals that ultimately favors an activation of Akt (42). Lastly, the chronic low-grade inflammatory state associated with obesity results in an elevation in proinflammatory cytokines in various tissue compartments, and these inflammatory mediators are capable of activating several well-known procarcinogenic signaling cascades including Akt (11). An apparent unifying characteristic of these three hypotheses is the Akt pathway. Insulin (8), leptin (37), and proinflammatory cytokines (9) all have been consistently demonstrated to signal through protein kinase B, also known as Akt. Thus, regardless of the particular upstream events, Akt activation and the various transcriptional consequences are likely to be an important molecular link between obesity and intestinal tumorigenesis. Akt has been studied extensively as a master regulator of critical cellular processes including growth, proliferation, and cell cycle control. In this regard, overactivation of Akt signaling has been identified consistently as a critical component of initiation, promotion,
and progression of human tumors (33). Furthermore, PI3K/Akt signaling has been found to be activated in human colorectal tumors, and evidence suggests that mutations could be involved in tumorigenesis and metastasis (13). Several Akt inhibitors show promise as chemotherapeutic agents (6), further demonstrating the importance of Akt in tumorigenesis.

It is becoming increasingly apparent that the gut microbiota is a major determinant of various aspects of health and disease, including tumor formation. Indeed, several reports have documented alterations in the gut microbiota in those harboring colonic adenomas and carcinomas (44). One mechanism by which the gut microbiota might affect tumor formation is by directly affecting cellular signaling. Recent studies estimate that ~10% of the colonic epithelial transcriptome may be sensitive to the microbial composition, with many of these genes having roles in immune modulation, proliferation, and cell cycle regulation (36). The ability of the microbiome to interact with the colonic epithelium has substantial implications for understanding the relationship between obesity and elevated CRC risk. Although it is well recognized that experimental obesity enhances tumorigenesis (7), it remains unclear whether it is the excess adiposity, consumption of high-fat diet, or some other factor associated with obesity that is responsible for the alteration in procarcinogenic signaling pathways that drive the process.

Studies have demonstrated that diet-induced obesity elevates intestinal tumorigenesis; however, saturated fats activate procarcinogenic signaling pathways, and thus it has been difficult to distinguish the effects of obesity per se from elevated saturated fat intake (1). Saturated fatty acids as well as n-6 polyunsaturated fatty acids are also known to induce an elevation in proinflammatory cytokines (40). This is particularly important in understanding the association between obesity and CRC, as one of the prevailing hypotheses posits that the chronic, low-grade inflammatory state associated with obesity is responsible for the elevation in cancer risk primarily through the ability of cytokines to stimulate protumorigenic cell signaling pathways such as Toll-like receptor signaling (5). As such, it is important to gain a better understanding of how different models of obesity differentially alter interactions between the colonic microbiome and the transcriptome and their potential impact on cellular programming and tumorigenesis.

METHODS

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. The Apcc1638N (National Cancer Institute Mouse Repository, Frederick, MD) strain of mice was used to study intestinal tumorigenesis. Apc1638N mice were maintained on a low-fat diet (10% calories from fat) or made obese either through the consumption of a high-fat diet (60% calories from fat) or the presence of the leptin receptor mutation (DbDb; Jackson Laboratory, Bar Harbor, Maine). A full description of the animal protocol has previously been reported (29). In brief, these three groups of mice were maintained for 16 wk and then euthanized, at which time tumor burden was documented. In addition, stool was collected for profiling the microbiome and metabolome from the same mice. Finally, after rinsing the colon with PBS, followed by PBS plus protease inhibitors (Roche, Indianapolis, IN), we collected the colonic mucosa by gentle scraping with glass microscope slides. Mucosa was frozen in liquid N2 and stored at −80°C for gene expression analyses. RNA was isolated from the colonic mucosa (n = 10 per group) using the Ambion RiboPure Kit (Life Technologies, Grand Island, NY) and quality assessed using the 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA) to verify the RNA integrity number was > 8. cDNA libraries were prepared with the TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA), with 1 μg of input RNA per sample. Quality was assessed using the Fragment Analyzer (Advanced Analytical, Ames, IA). Single-end sequencing was performed on the HiSeq 2500 (Illumina). The demultiplexed FASTQ files were generated using CASAVA 1.8.2 (Illumina), and the quality control reports were generated with FastQC. Samples were considered of acceptable quality if the mean quality score was at least 30 and the percentage of bases with a quality score of ≥ 30 was at least 85%.

TopHat and Cufflinks programs within the Tuxedo Suite programming package were used as the primary method of RNA sequencing (RNA-Seq) analysis. The RNA-Seq reads from the FASTQ files were aligned to the mouse genome (mm10, GRCm38) using TopHat (http://ccb.jhu.edu/software/tophat/index.shtml) version 2.0.11. The output read alignments contained in BAM files were used by Cufflinks (http://cufflinks.cbcb.umd.edu/index.html) version 2.2.1 to assemble transcripts. BAM files from TopHat output were converted to SAM files using Samtools, available from http://www.htslib.org/. HTSeq-count, an open-source tool available from http://www-huber.embde.de/HTSeq, was used to count the number of reads mapping to each gene. DESeq2, an open-source Bioconductor package, was then used in R to identify differentially expressed genes (19). DESeq2 estimates the effect size by calculating the log2 fold-change of the “treatment” sample compared with control. A false discovery rate with a cut-off of q ≤ 0.10 was used for determining differential expression in the analysis of the colonic transcriptome. Comparisons of gene lists from both obese conditions, low-fat diet (LF) vs. high-fat diet (HF) and LF vs. DbDb, were done using VENNY, an online Venn diagram tool (26) that identified genes common to both data sets.

Ingenuity Pathway Analysis (IPA; Qiagen, Redwood City, CA) was used to identify functional categories that were enriched as well as networks of interacting genes within the lists of differentially expressed genes (q < 0.10) obtained from the DESeq2 analysis of two comparisons (LF vs. HF and LF vs. DbDb). Top diseases and functions were identified based upon a network score generated from a hypergeometric distribution and calculated with the right-tailed Fisher’s exact test. For instance, a network composed of 35 molecules with a Fisher’s exact test of 1 × 10−6 has a network score of −log(Fisher’s exact test) = 6. This network score can be interpreted as there being one in a million chance of observing a network containing the same number of molecules by chance when randomly picking 35 molecules (30).

The weighted correlation network analysis (WGCNA) (14) algorithm, in R/Bioconductor (31), was used to identify gene coexpression modules. In the construction of the weighted gene network, a soft thresholding power was selected based on the approximate free topology described in detail elsewhere (45). For each gene, WGCNA quantifies module membership as the correlation of the module eigengene and its corresponding expression profile. Correlations between gene expression modules with gut microbial taxa abundance and fecal metabolites from the same samples were determined.

Six genes found to be differentially expressed by RNA-Seq were selected for validation by RT-PCR. cDNA synthesized using SuperScript III reverse transcriptase (Invitrogen, Grand Island, NY) from RNA extracted above. Real-time PCR was performed with TaqMan probe-based assays for Creb3l3, Mfsge8, Fابp5, and Rbp1 on a QuantStudio 6 Flex (Applied Biosystems, Foster City, CA). Gapdh was used as the control gene. Relative expression was calculated by the 2−ΔΔCt method, and statistical analyses were performed on ΔCt values. All data are reported as means ± SE. Statistical calculations were performed in Systat (San Jose, CA) and GraphPad Prism (La...
Differential expression was determined with DESeq2, and significance was set using a per sample (range: 14.2–41.2) t-test. A similar significant step-wise increase in tumor multiplicity and burden was also observed (29).

The online version of this article contains supplemental material.

RESULTS

Physiological, tumor, microbiome, and metabolome data have been reported previously (29). In brief, both genetically induced (DbDb) and diet-induced (HF) mice had significantly greater body weight compared with LF-fed mice. Fat mass was significantly higher in DbDb mice compared with HF- and LF diet-fed mice and HF diet-fed mice had significantly greater body fat than LF diet-fed mice (29). Tumor incidence was 33, 67, and 100% in LF, HF, and DbDb mice, respectively (P < 0.005). All tumors were histologically confirmed to be adenomatous polyps. A similar significant step-wise increase in tumor multiplicity and burden was also observed (29).

An average of 18,043,084 single-end reads were retrieved per sample (range: 14.2 – 41.2 × 10^6). Reads were aligned to 23,338 mouse reference mRNAs using the mouse genome build, mm10. Compared with the lean group, 266 (157 upregulated and 109 downregulated) and 584 (308 upregulated and 276 downregulated) differentially expressed genes were detected in HF and DbDb mice, respectively, using DESeq2 (Q < 0.10) (Fig. 1, A and B, and Supplemental Tables S1 and S2). Common to both comparisons were 74 significantly differentially expressed genes, and each was altered in the same direction.

Lists of differentially expressed genes were analyzed with Ingenuity Pathway Analysis (IPA) to identify enrichment of specific canonical pathways, biological functions, and networks of interacting genes. For the HF effect, 157 genes were assigned to pathways by IPA, and the top five canonical pathways identified were related to immune function as well as cell death and survival (Table 1). Among the top five cellular functions were cellular growth and proliferation and cell-to-cell signaling and interaction. The most commonly attributed diseases for genes differentially expressed in HF mice were endocrine disorders, gastrointestinal and immunological diseases, as well as disorders related to inflammatory response (Table 1). For the DbDb effect, 345 genes were assigned to pathways by IPA, and the top five pathways were also related to immunity, while lipid metabolism was identified as another prominent network. The top cellular functions were associated with cellular function and maintenance, and lipid metabolism. The top diseases identified were cardiovascular disease and disorders associated with inflammatory response (Table 2).

Interestingly, although not differentially expressed in our dataset, protein kinase B (Akt) was a central component of the top network in both comparisons (Fig. 2, A and B). Moreover, several known regulators of Akt were differentially expressed in both obese comparisons: Retinol binding protein (Rbp1), CAMP responsive element binding protein 3 like-3 (Creb3l3), Milk fat globule EGF factor 8 (Mfge8), and Fatty acid binding protein 5 (Fabp5). PI3K signaling and forkhead-box O4 (Foxo4) identified within the top network for the DbDb comparison were also of interest as they are known Akt regulators. These six genes were chosen for validation by RT-PCR. Creb3l3 and Rbp1 were shown to be downregulated ~60% in HF and DbDb by both RNA-Seq and RT-PCR. Mfge8 and Fabp5 had significantly elevated expression in both HF and DbDb comparisons, consistent with RNA-Seq data. Foxo4 was found to be significantly upregulated by RNA-Seq and RT-PCR in both DbDb and HF colon, while Pik3r5 was only significantly elevated in the DbDb colon, a change verified by RT-PCR (Fig. 3).

To identify putative pathways by which the colonic microbiome and metabolome might modulate tumorigenesis, we analyzed their association with gene expression modules via weighted coexpression correlation analysis (14). For the microbiome analysis, WGCNA identified 15 gene modules that significantly associated with at least one of 16 bacterial taxa (Fig. 4A). Among these taxa, Clostridium (R = 0.58, P < 0.01), Sarcina (R = 0.33, P = 0.03), and Rikenellaceae (R = −0.28, P = 0.07) were previously found to be associated with tumor burden in these same mice (29). Interestingly, the “grey60” gene module was negatively associated with the two genera (Clostridium and Sarcina) that were positively associ-
ated with tumor burden, while it was positively associated with one family (Rikenellaceae) that was negatively associated with tumor burden (29). The average relative abundance (and range) of genera *Clostridium* and *Sarcina* and family Rikenellaceae was 0.001% (0 – 0.007%), 0.004% (0 – 0.04%) and 7.6% (0.2–20.2%), which at least for the latter taxa is clearly a biologically significant proportion. The grey60 module contains 206 genes, and pathway analysis revealed that the top disease category associated with this gene set was cancer (151 genes; $P = 8.5 \times 10^{-3} – 1.8 \times 10^{-2}$). Of note, the highest scoring network (score = 41) of genes pertained to "cellular growth and proliferation, cellular development, hematological system development and function" and focused on Akt (Fig. 4B).

For the metabolite analysis, WGCNA identified 12 gene modules that significantly associated with at least one of 54 stool metabolites (Fig. 5A). Among these metabolites, we previously found adenosine concentrations measured from

<table>
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<tr>
<td>Cortisol</td>
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<td>Gastrointestinal disease</td>
<td>1.33E-03–3.97E-29</td>
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<tr>
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<td>7.14E-04–3.97E-29</td>
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<td>Metabolic response</td>
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Molecular and Cellular Functions

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<td>Protein synthesis</td>
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Networks

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<tr>
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<tr>
<td>endocrine system disorders, gastrointestinal disease, metabolic disease</td>
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<td>cellular function and maintenance, hematological system development and function, cell death and survival</td>
<td>31</td>
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<tr>
<td>hematological system development and function</td>
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HF, high-fat diet; LF, low-fat diet.

<table>
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<td>Organismal injury and abnormalities</td>
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Molecular and Cellular Functions

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Networks

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<td>cardiovascular disease, connective tissue disorders, cancer</td>
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<td>cellular development, cellular growth and proliferation, embryonic development</td>
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DbDb, mice that are genetically obese through leptin receptor mutation.

**Table 1. Top 5 diseases, functions, and networks associated with genes differentially expressed in HF vs. LF mice**

**Table 2. Top 5 diseases, functions, and networks associated with genes differentially expressed in DbDb vs. LF mice**
stool to be significantly depleted in obese and tumor-bearing mice and also to be inversely associated with colonic Tnfa and Il1b concentrations (29). In this analysis adenosine was found to be associated with two gene expression modules [Fig. 5A: “royal blue” (948 genes; \( P < 0.001 \)) and “green” (1,907 genes; \( P < 0.001 \))]. Pathway analysis of the royal blue gene set found that the top disease category was cancer (702 genes; \( P = 4.7 \times 10^{-3} \)), and the top function associated with these genes is “cellular growth and proliferation” (295 genes; \( P = 4.59 \times 10^{-3} \)). Pathway analysis of the green gene set also found the top disease category to be cancer (1,418 genes; \( P = 1.91 \times 10^{-3} \)), while the top functional category associated with these genes was lipid metabolism (324 genes; \( P = 1.9 \times 10^{-3} \)). Relevant to tumorigenesis, the second highest scoring network in the green module (score \( 37 \)) pertains to “cellular assembly and organization, amino acid metabolism, developmental disorder” and again focused on Akt (Fig. 5B).

As previously described (29), we also tested the relationship between fecal operational taxonomic units (OTUs) and metabolites; 107 metabolites were related to at least one OTU, and 31 OTUs were related to at least one metabolite (\( q < 0.05 \)).

DISCUSSION

In this study, we demonstrate that the induction of obesity with both an HF diet and mutation of the leptin receptor (DbDb) promotes extensive changes in the colonic mucosal transcriptome. Of note, the number of genes with altered expression, as well as the number of intestinal tumors, was markedly higher in DbDb animals compared with HF-fed animals. The greater body weight and fat mass in the DbDb animals compared with the HF-fed animals (29) is a more profound perturbation to homeostasis and may partially explain this difference. Additionally, it is possible that the more numerous transcriptional changes in DbDb mice may result from...

Fig. 2. Pathway analysis of genes differentially expressed in diet and genetically induced obese mice. A: top-scoring Ingenuity Pathway Analysis (IPA) network among HF-induced changes is associated with “humoral immune response, protein synthesis, cellular function and maintenance.” B: top-scoring network for the top-scoring IPA network among DbDb-induced changes is associated with “cellular function and maintenance, cell-to-cell signaling and interaction, cellular movement.” Gene names written in ovals were differentially expressed (\( q \) value < 0.10) in our transcriptional analysis, and genes and/or protein complexes in rectangles were not included in transcriptome analysis. Genes in red ovals indicate an upregulation, and genes in green ovals indicate a downregulation in obese mice compared with lean. Shades of color indicate the degree of fold-change.

Fig. 3. Validation of gene expression changes. Real-time PCR validation of genes identified to be differentially expressed in both HF and DbDb mice compared with lean animals and known regulators of Akt. Data represents means ± SE. Bars with different letters are significantly different (\( P < 0.05 \)). *\( P \) value = 0.08 vs. LF.
the direct effects of impaired leptin signaling as leptin is a known transcriptional regulator, primarily through the activation of the STAT transcription factor as well as regulation of AMPK activity (24). Interestingly, although the transcriptional response to obesity varies significantly by model, the most prominent physiological consequence for both obese comparisons, as identified by pathway analysis, is related to immune function and inflammatory response. These findings agree with previous reports suggesting that obesity blunts the immune response and often results in low-grade biochemical inflammation in various tissues (22). Although our transcriptional analyses did not suggest that proinflammatory cytokines were differentially expressed in the colon, changes at the protein level remain possible.

The patterns of genes altered in both obese groups suggests that the pathways altered in obesity per se are likely regulated by Akt (Fig. 2). Protein kinase B, also known as Akt, is a central “hub” upon which numerous signaling pathways converge (2). These signaling pathways regulate a variety of biological processes making Akt a central regulator of many cellular functions, including cellular proliferation, apoptosis, growth, and differentiation (2). Consistent with these regulatory functions is the fact that changes in Akt activity consistently have been associated with several human cancers (6). Certain genes identified by our transcriptome analysis are known to be altered in obese models and known Akt regulators are potential mechanisms by which obesity may be elevating intestinal tumorigenesis. For instance, fatty-acid binding protein 5 (Fabp5) was upregulated threefold in HF and fourfold in DbDb models. FABPs are expressed in various tissues and are involved in energy metabolism; however, Fabp5 has been specifically linked to cancer as it has been shown to regulate PPAR transcriptional activity and proliferation (41). Milk fat globule EGF factor 8 (Mfg8), also known as lactadherin, was also significantly upregulated in both obese models. Mfge8 facilitates phagocytosis of apoptotic cells and is consistently upregulated in various tumors compared with normal tissue (25). Retinol binding protein 1 (Rbp1) belongs to the same family of fatty-acid binding proteins as FABP5 (4) and was downregulated in both obese models. Mfge8 facilitates phagocytosis of apoptotic cells and is consistently upregulated in various tumors compared with normal tissue (25). Retinol binding protein 1 (Rbp1) belongs to the same family of fatty-acid binding proteins as FABP5 (4) and was downregulated in both obese models. Mfge8 facilitates phagocytosis of apoptotic cells and is consistently upregulated in various tumors compared with normal tissue (25).
in both HF and DbDb animals, typically has been inversely associated with tumor growth and proliferation (38).

Our study also investigated the interaction of fecal microbes and metabolites with modules of coexpressed colonic transcript sequences. It has been estimated that ~10% of the mouse epithelial transcriptome is regulated by the gut microbiota (36). Our analysis identified 16 bacterial taxa that were significantly associated with at least one gene module (Fig. 4A). Of interest, the genera Clostridium and Sarcina, both of which were positively associated with tumor burden, were negatively correlated with the grey60 gene module. Conversely, the family Rikenellaceae, which was negatively associated with tumor burden, were negatively correlated with this gene set. The taxon Rikenellaceae was positively correlated with the grey60 gene module. Conversely, the family Rikenellaceae, which was negatively associated with tumor burden, were negatively correlated with the grey60 gene module. Conversely, the family Rikenellaceae, which was negatively associated with tumor burden, were negatively correlated with the grey60 gene module. Conversely, the family Rikenellaceae, which was negatively associated with tumor burden, were negatively correlated with the grey60 gene module.

We also considered associations between fecal metabolites and the colonic transcriptome. Fifty-four metabolites were significantly correlated with adenosine. 1, 1-oleoylglycerophosphoglycerol; 2, 1-stearoylglycerophosphoinositol; 3, 1,3-methylmyristic acid; 4, 2-aminoobutyrate; 5, 3-dehydroascorbate; 6, 3-dehydrocholate; 7, 3-dehydroxypropanoate; 8, 4-hydroxyoctanoate; 9, 8-hydroxyoctanoate; 10, adenosine; 11, adenosine-2,3-cyclic monophosphate; 12, adrenate; 13, alanine; 14, alanyltyrosine; 15, alanylleucine; 16, azelate; 17, beta-alanine; 18, carnitine; 19, cholate; 20, cholesteryl; 21, cis-vaccenate; 22, citrate; 23, cyclo(leu-phe); 24, cysteine; 25, delta-tocopherol; 26, diaminopimelate; 27, dodecanedioate; 28, gamma-tocopherol; 29, glutamine; 30, guanosine-2,3-cyclic monophosphate; 31, octadecenoic acid; 32, isoleucylmethionine; 33, isoleucyltyrosine; 34, L-urobilin; 35, lithocholate; 36, N-acetylglucosamine; 37, octadecanediol; 38, prolyltyrosine; 39, serylleucine; 40, serylphenylalanine; 41, spermidine; 42, suberate; 43, taurine; 44, threonylmethionine; 45, undecanedioate; 46, uridine; 47, uridine-2,3-cyclic monophosphate; 48, val-val-val; 49, valylisoleucine; 50, valyleucine; 51, valylmethionine; 52, valylphenylalanine; 53, valylvaline. B: IPA of the "green" module identified the top disease category as "cancer" and Akt was the central target of genes within this network.

We also considered associations between fecal metabolites and the colonic transcriptome. Fifty-three metabolites were associated with at least one gene module (Fig. 5A). Among these, adenosine is of particular interest because as it was negatively associated with proinflammatory cytokines IL1b and TNfa and also with tumor burden (29). Adenosine was most strongly associated with royal blue and green modules. Again, there was an enrichment of cancer-related genes in both of these modules and network analysis built networks focusing on Akt (Fig. 5B).

Akt activation is a multistep process that occurs posttranslationally and involves the activation of several effector molecules that phosphorylate Akt at Ser473 and Thr308 (17). Indeed, in several in vitro and in vivo models, stimulation of the PI3K/Akt pathway results in a robust increase in Akt phosphorylation (18, 20, 43). However, it is technically challenging to detect phosphorylation of Akt at either residue in an unstimulated or otherwise healthy tissue. In fact, previous investigators measuring Akt activity in "normal" cells/tissue often either use indirect markers of Akt phosphorylation such as carboxy-terminal modulator protein (21) or stimulate their samples to induce a more robust activation of Akt (10). Thus, despite using several commercially available antibodies to phosphor Akt we were unable to detect a robust signal in our normal tissue with Western blotting.
Another limitation of this study is the mouse model employed, like most other genetically engineered models (32), develops tumors in the small rather than large intestine. The biochemical and molecular milieu of the colon differs from that of the small intestine, and since the clinical translation of this study ultimately pertains to how obesity affects the colonic environment and its prediction toward carcinogenesis via the interactions between the colonic microbiome, metabolome, and transcriptome, the large, rather than small, intestinal transcriptome was examined. Although this does create a degree of disconnect between our tumor and transcriptome endpoints, it does not negate the import of either observation.

In summary, we have attempted to gain a better understanding of the mechanisms by which obesity promotes CRC by comparing colonic gene expression signatures of diet and genetically induced obese mice. A modest degree of overlap was observed for these models: 74 genes were altered in both comparisons, comprising 27 and 12% of altered genes in HF and DbDb mice, respectively. Among these common genes, we confirmed expression changes for several regulators of Akt. Moreover, networks focusing on Akt were the most robust found within each gene set. To assess the possible influence of the gut microbiome and metabolome on gene expression, we performed coexpression analyses. Several gene modules were significantly associated with numerous taxa and metabolites. Of particular interest were modules that associated with metabolites and taxa that were previously associated with tumor burden in these same mice. Pathway analyses of these modules again highlighted Akt signaling, with the strongest network of genes for each module once again focusing on Akt. These data suggest that although HF feeding and genetic obesity have distinct effects on the colonic transcriptome, there is considerable overlap and Akt appears to be a common target for both perturbations. Likewise, we have identified specific metabolites and microbes that could mediate the activation, or repression, of Akt in obesity and as such are potential targets for CRC prevention in the obese. Clearly further work is required to establish whether microbiota and metabolite regulation of Akt in obesity is causal in the observed increase in tumorigenesis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


