

Obesity promotes conserved inflammatory and metabolic transcriptional programs in colon tumors: evidence from mouse models and the ColoCare Study Patient Cohort

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Abstract

Background: The global prevalence of obesity, an established risk and progression factor for colon cancer, is high and rising. Unfortunately, the mechanisms underlying the obesity–colon cancer association are incompletely understood, and new molecular targets enabling more effective intervention strategies to break the obesity–colon cancer link are urgently needed.

Objectives: This study integrated RNA sequencing data from mouse and human colon tumor samples, as well as human adipose samples, to rigorously establish obesity-associated transcriptomic signatures conserved between the 2 species.

Methods: We employed a mouse colon cancer model with colonoscopy-guided orthotopic transplantation of syngeneic Apcnull KrasG12D/+ Trp53null Smad4null tdTomato colon tumor organoids. Epithelial cell adhesion molecule (EpCAM)-positive cells from murine tumors and 193 human colon tumors and 188 human mesenteric adipose tissue samples from the prospective ColoCare Study cohort underwent transcriptomic analyses.

Results: Diet-induced obesity reduced survival in the mouse model of colon cancer. Integrated transcriptomic analyses of EpCAM-positive murine tumor cells and bulk human tumors revealed obesity-driven enrichment of inflammation and metabolic pathways, including the upregulation of genes involved in innate immune sensing (TLR2, MYD88, and

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IRF4) and tumor microenvironment remodeling (MMP9, TGFB1, and SERPINE1). Analysis of paired mesenteric visceral adipose tissue and tumor samples from the study patients (63 ± 13 y, 48% female, body mass index 28.9 ± 6.0 kg/m²) indicated that obesity was associated with enriched inflammatory signaling pathways through unique adipose ligand-tumor receptor interactions.

Conclusions: These results establish obesity-associated adipose tissue dysregulation as a key intertissue modulator of biology, with concordant cross-species effects on tumor cell-intrinsic inflammatory and metabolic programs.

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Original Research Article

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ABSTRACT

Background: The global prevalence of obesity, an established risk and progression factor for colon cancer, is high and rising. Unfortunately, the mechanisms underlying the obesity–colon cancer association are incompletely understood, and new molecular targets enabling more effective intervention strategies to break the obesity–colon cancer link are urgently needed.

Objectives: This study integrated RNA sequencing data from mouse and human colon tumor samples, as well as human adipose samples, to rigorously establish obesity-associated transcriptomic signatures conserved between the 2 species.

Abbreviations: AKPST, *Apc*^{null} *Kras*^{G12D/+} *Trp53*^{null} *Smad4*^{null} *tdTomato*; DIO, diet-induced obesity; EpCAM, epithelial cell adhesion molecule; FC, fold change; FDR, false discovery rate; GSEA, gene set enrichment analysis; HCI, Huntsman Cancer Institute; MSigDB, Molecular Signatures Database; ORA, overrepresentation analysis; RNA-Seq, RNA sequencing; STAR, Spliced Transcripts Alignment to a Reference; TLR, toll-like receptor; VAT, visceral adipose tissue.

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Methods: We employed a mouse colon cancer model with colonoscopy-guided orthotopic transplantation of syngeneic *Apc^{mut} Kras^{G12D/+} Trp53^{null} Smad4^{null} tdTomato* colon tumor organoids. Epithelial cell adhesion molecule (EpCAM)-positive cells from murine tumors and 193 human colon tumors and 188 human mesenteric adipose tissue samples from the prospective ColoCare Study cohort underwent transcriptomic analyses.

Results: Diet-induced obesity reduced survival in the mouse model of colon cancer. Integrated transcriptomic analyses of EpCAM-positive murine tumor cells and bulk human tumors revealed obesity-driven enrichment of inflammation and metabolic pathways, including the upregulation of genes involved in innate immune sensing (*TLR2*, *MYD88*, and *IRF4*) and tumor microenvironment remodeling (*MMP9*, *TGFBI*, and *SERPINE1*). Analysis of paired mesenteric visceral adipose tissue and tumor samples from the study patients (63 ± 13 y, 48% female, body mass index 28.9 ± 6.0 kg/m²) indicated that obesity was associated with enriched inflammatory signaling pathways through unique adipose ligand–tumor receptor interactions.

Conclusions: These results establish obesity-associated adipose tissue dysregulation as a key intertissue modulator of biology, with concordant cross-species effects on tumor cell–intrinsic inflammatory and metabolic programs.

Keywords: obesity, colon cancer, visceral adipose tissue, inflammation, metabolism, RNA sequencing, ligand–receptor interactions

Introduction

Colorectal cancer is the third most common cancer and the second leading cause of cancer deaths worldwide, with ~1.9 million new cases, 900,000 deaths in 2022, and a rising early-onset rate [1–3]. Obesity, a metabolic disease defined as a BMI ≥ 30 kg/m², is a major risk and prognostic factor for colon cancer and ≥ 12 other cancer types [4]. Patients with colon cancer and obesity have reduced overall survival and disease-free survival risk relative to patients with a normal BMI (< 25 kg/m²) [5]. Obesity rates have tripled worldwide since 1975, and $> 70\%$ of adults in the United States are either overweight or obese [6,7]. However, critical biological pathways underpinning the obesity–colon cancer relationship remain incompletely understood, hampering efforts to develop effective mechanism-based nutritional or pharmacologic interventions to reduce the burden of obesity-driven colon cancer [8].

Expanded and dysregulated visceral adipose tissue (VAT) is a hallmark of obesity [8–11]. In colon cancer, mesenteric VAT envelopes the colonic serosa and interfaces with the tumor vasculature, facilitating the exchange of adipose-derived factors. Beyond its role as an energy storage site, VAT serves as an active endocrine tissue comprised of many cell types, including adipocytes, immune cells, and fibroblasts that collectively secrete proteins and metabolites involved in the regulation of energy homeostasis and metabolic and inflammatory pathways [8, 11]. Altered morphological and functional changes in VAT, as well as levels of several VAT-derived factors (particularly adipokines, cytokines, growth factors, and lipid mediators) may contribute to the enhancing effects of obesity on colon cancer risk and progression [8,9, 11]. However, actionable targets to disrupt the crosstalk between obesity-associated dysregulated VAT and the colon tumor have yet to be systematically identified and characterized.

Murine models are instrumental for deeply interrogating the mechanistic underpinnings of cancer, but they do not always recapitulate the biological complexities of human disease [12–15]. Conversely, observational studies using human samples are invaluable for identifying candidate genes and pathways highly relevant to cancer development and progression but are typically limited to identifying associations rather than establishing causal relationships [16]. When evaluated in parallel, however, preclinical and human data synergistically inform each other to accelerate the pace of translational research on obesity and cancer [17–20]. Hence, our primary outcome was the identification of obesity-associated transcriptional networks conserved across human and mouse colon cancer, with exploration of VAT-to-tumor paracrine signaling networks as an important secondary outcome.

Herein, we leveraged transcriptomic data from both preclinical and human studies to identify putative molecular targets for

disrupting the obesity–colon cancer link, as summarized in Figure 1. Specifically, through an integrated genomics analysis using a state-of-the-art obesity-driven orthotopic colon cancer mouse model and paired tumor and mesenteric VAT samples from an international cohort of patients with newly diagnosed colon cancer [21], we identify obesity-responsive pathways in tumors, interactions between dysregulated VAT and the colon tumor, and pro-cancer gene candidates conserved between species. These analyses disclose new molecular targets for disrupting obesity-driven colon cancer that are conserved across 2 species and reveal VAT-to-tumor paracrine signaling as a putative driver of inflammatory processes in the tumor.

Methods

Study approval

All human study participants provided written informed consent, and the research was approved by the institutional review boards of all the participating institutions. All mouse studies were approved by the Institutional Animal Care and Use Committee at Duke University.

Study population

The study population was drawn from the international prospective ColoCare Study cohort (U01CA206110; clinicaltrials.gov identifier: NCT02328677) [21]. This multicenter cohort study recruited male and female adult patients (aged 18–89 y) with primary invasive colorectal cancer who were scheduled to undergo surgical intervention at participating research centers. Exclusion criteria included those with insufficient language or consent capacity, women who are pregnant, and prisoners. Patient-reported race was assessed by a questionnaire asking “Which of the following best describes your racial background?,” with response options listed as: White; Black or African American; American Indian or Alaska Native; Asian; Pacific Islander; Other; More than One; or Refuse to Answer. As illustrated in the flow diagram (Supplemental Figure 1) and clinicodemographic data (Table 1, Supplemental Table 1), neoadjuvant-naïve patients with stage I–III colon cancer with flash-frozen colon tumor tissue and tumor-adjacent VAT were eligible for the current study. Biospecimens were collected irrespective of other characteristics. Included in these analyses, based on biospecimen availability at participating sites in the United States [Huntsman Cancer Institute (HCI), Moffitt Cancer Center, University of Tennessee Health Science Center] and Germany (Heidelberg University Hospital), eligibility criteria, sample quality, and funding availability, were 93 male and 100 female neoadjuvant-naïve patients enrolled in the ColoCare Study between April 2011 and July 2021 with flash-frozen colon tumor samples available.

Among them, 188 patients had sufficient flash-frozen tumor-adjacent VAT for RNA sequencing (RNA-Seq) analysis and only 1 patient had a BMI <18.5 kg/m².

Animals and diets

Female C57BL/6Hsd mice were purchased from Inotiv (#044) and housed in a temperature- and humidity-controlled specific-pathogen-free facility with a 12-h light cycle. Cages contained ALPHA-dri bedding (Shephard Specialty Papers), and mice were randomly assigned to be provided with ad libitum access to either a control (10% kcal fat) purified diet (D12450J; Research Diets, Inc) or a diet-induced obesity (DIO; 60% kcal fat) purified diet (D12492; Research Diets, Inc) for the duration of the studies. At study termination, mice were anesthetized using 500 mg/kg 2,2-tribromoethanol and killed via thoracotomy when unresponsive to toe pinch.

Organoid orthotopic transplantation

Dissociated *Apc*^{null} *Kras*^{G12D/+} *Trp53*^{null} *Smad4*^{null} *tdTomato* (abbreviated AKPST) organoids (a kind gift from Ömer H. Yilmaz, Massachusetts Institute of Technology) were mixed with Matrigel (#356231; Corning) at a 1:3 ratio. Seven or eight 20- μ L domes were

plated in a 6-well plate and incubated at 37°C for 10–15 min. After Matrigel polymerization, organoid media [Advanced DMEM-F12 media (Gibco) supplemented with 5% fetal bovine serum (Cytiva), 2% B27 (Gibco), 1% L-glutamine (Gibco), and 1% antibiotic-antimycotic (Gibco)] was added, and cultures were maintained at 37°C with 5% CO₂. Organoids were passaged every 2–3 d using cell recovery solution (Corning) to dissolve the Matrigel and TrypLE express enzyme (Gibco) to create a single-cell suspension before reseeding.

AKPST organoids were orthotopically transplanted as previously described for other colon cancer organoid lines [22,23]. Briefly, Matrigel was chemically dissociated using 500 μ L cell recovery solution for 30 min on ice. Organoids were pelleted, washed with cold phosphate-buffered saline, and resuspended in 10% Matrigel with organoid media containing no fetal bovine serum at a concentration of 10 organoids/ μ L. Mice were anesthetized with 1%–2% isoflurane, placed in the supine position, and kept warm on a heating pad for the procedure (SomnoSuite, Kent Scientific). Colons were flushed with water using a gavage needle to expunge fecal matter. Mice were injected with a single 70- μ L bolus of AKPST organoids into the colonic submucosa via colonoscopy-guided injection (Coloview, Karl Storz) using a 16 in, 45° bevel, 33-gauge needle (Hamilton).

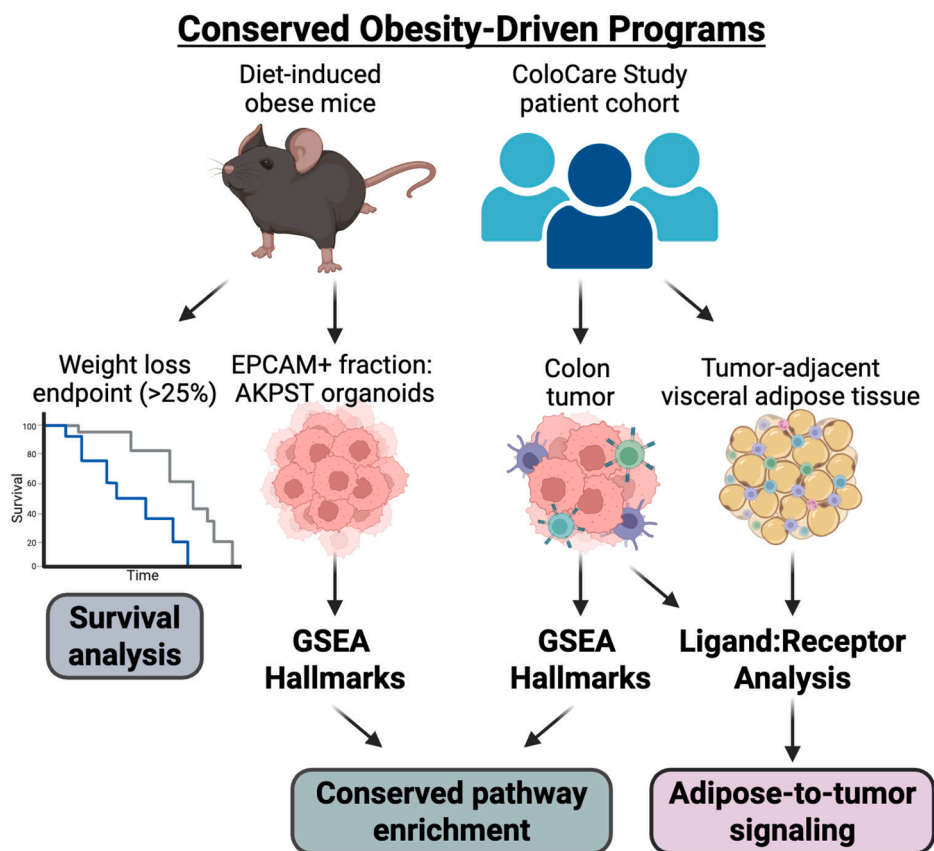


FIGURE 1. Research design and analytical approach to identify shared obesity-responsive transcriptional programs in preclinical and clinical colon cancer specimens. EPCAM⁺ fraction refers to epithelial cell adhesion molecule (EpCAM)-positive colon tumor cells isolated from murine *Apc*^{null} *Kras*^{G12D/+} *Trp53*^{null} *Smad4*^{null} *tdTomato* (abbreviated AKPST) organoids. Gene set enrichment analysis (GSEA) after RNA sequencing of the murine EPCAM⁺ fractions, human colon tumors, and human colon tumor-adjacent visceral adipose tissue was used to assess conserved pathway enrichment across the 2 species and conduct ligand-receptor analysis. Created in [BioRender.com](https://www.biorender.com).

TABLE 1
Baseline clinicodemographic characteristics of study patients, by BMI category and overall¹

	Normal BMI (n = 45)	Overweight BMI (n = 82)	Obese BMI (n = 66)	Overall (n = 193)
Age at diagnosis (y)				
Mean \pm SD	64.7 \pm 13.7	63.2 \pm 13.4	62.0 \pm 12.1	63.1 \pm 13.0
Median (min, max)	68.0 (19.0, 86.0)	64.5 (18.0, 86.0)	62.5 (27.0, 84.0)	64.0 (18.0, 86.0)
Sex				
Female	32 (71.1%)	27 (32.9%)	34 (51.5%)	93 (48.2%)
Male	13 (28.9%)	55 (67.1%)	32 (48.5%)	100 (51.8%)
Race				
White	43 (95.6%)	74 (90.2%)	58 (87.9%)	175 (90.7%)
Black or African American	0 (0%)	6 (7.3%)	5 (7.6%)	11 (5.7%)
American Indian or Alaska Native, Asian, or Pacific Islander	0 (0%)	2 (2.4%)	3 (4.5%)	5 (2.6%)
Other	2 (4.4%)	0 (0%)	0 (0%)	2 (1.0%)
Presurgery BMI ² (kg/m ²)				
Mean \pm SD	22.4 \pm 1.6	27.4 \pm 1.3	35.1 \pm 5.5	28.9 \pm 6.0
Median (min, max)	22.4 (18.0, 24.9)	27.4 (25.1, 29.8)	33.4 (30.0, 59.1)	28.0 (18.0, 59.1)
Tumor stage at diagnosis				
I	9 (20.0%)	13 (15.9%)	4 (6.1%)	26 (13.5%)
II	15 (33.3%)	35 (42.7%)	29 (43.9%)	79 (40.9%)
III	21 (46.7%)	34 (41.5%)	33 (50.0%)	88 (45.6%)
Microsatellite instability status testing				
Microsatellite stable	26 (66.7%)	50 (72.5%)	45 (78.9%)	121 (73.3%)
Microsatellite unstable	13 (33.3%)	19 (27.5%)	12 (21.1%)	44 (26.7%)
Missing	6	13	9	28

¹ On the basis of 193 neoadjuvant-naïve patients with colon stage I–III cancer with tumor samples available ($n = 89$ from Huntsman Cancer Institute; $n = 65$ from Heidelberg University Hospital; $n = 20$ from University of Tennessee Health Science Center; $n = 19$ from Moffitt Cancer Center). Of these, 188 patients had adequate visceral adipose tissue samples for RNA sequencing analysis.

² BMI at cancer diagnosis or at enrollment.

Colonoscopy-guided orthotopic transplantation of colon tumor organoids into the colonic submucosa faithfully recapitulates key features of colon cancer such as anatomical localization, histological architecture, and immune infiltration, and thereby maximizes the translational potential of our findings [22].

Survival analysis

Eight-week-old Control and DIO female C57BL/6Hsd mice were maintained on either the 10 kcal% fat control diet or 60 kcal% fat DIO diet, respectively, for 35 wk to promote excessive weight gain and VAT accumulation in DIO mice. All mice were weighed immediately before orthotopic transplantation of 700 AKPST organoids in the colonic submucosa. Mice were weighed in a random order, and orthotopic transplant occurred when mice were 44–46 wk of age. Survival was defined as the number of days post orthotopic transplant that mice were alive until >25% unintentional weight loss occurred.

EpCAM+ enrichment from orthotopic colon tumors

Tumors were collected 4 wk after organoid transplantation into mice 32–33 wk of age, cut into 2- to 4-mm pieces, and stored on ice in Leibovitz's L-15 media (Corning). Tumors were dissociated into single-cell suspensions using a mouse tumor dissociation kit (Miltenyi Biotec) and a gentleMACS dissociator (Miltenyi Biotec). Resulting suspensions were sequentially filtered using 70- μ m and 30- μ m filters, centrifuged, and resuspended in RPMI 1640 media (Gibco). CD45⁺ cells were removed using CD45 MicroBeads (Miltenyi Biotec), and EpCAM⁺ cells were subsequently enriched from the CD45⁻ fraction using CD326 (EpCAM) MicroBeads (Miltenyi Biotec) per manufacturer's instructions. Cell fractions were centrifuged and pelleted cells were lysed with lysis buffer (Qiagen) and stored at -80°C .

RNA-Seq analysis for murine tumors

RNA was isolated from EpCAM⁺-enriched cell populations using the RNeasy Micro Kit (Qiagen). RNA was quantified on a Qubit 2.0 fluorometer (Life Technologies), and integrity was assessed on a TapeStation 4200 (Agilent Technologies). ERCC RNA Spike-In Mix (Thermo Fisher Scientific) was added to normalize total RNA. RNA-Seq libraries were prepared using a NEBNext Ultra II RNA Library Prep Kit (New England Biolabs). mRNAs were enriched, fragmented, and used for cDNA synthesis. Before sequencing, cDNA fragments were end-repaired, adenylated, and ligated with universal adapters, and libraries were quantified by a Qubit 2.0 fluorometer and validated on a TapeStation 4200. Sequencing was performed on an Illumina HiSeq instrument using a 2×150 bp paired-end configuration at a depth of >25 million reads per sample.

Raw data were converted to fastq files and demultiplexed using Illumina's bcl2fastq 2.17 software, allowing 1 mismatch for index sequence identification. Adapters were trimmed and low-quality reads were filtered using Trimmomatic [24]. Spliced Transcripts Alignment to a Reference (STAR) aligned reads to the reference GRCm38 mouse genome and produced a counts matrix [25]. Low-abundance genes (<10 read counts in ≥ 7 samples) were removed from all downstream analyses, and a normal shrinkage estimator was applied to log₂ fold change (log₂FC) estimates to enhance gene stability, particularly for genes with low counts and/or high variability [26]. Principal coordinates analysis plots were generated using the top 500 most differentially expressed genes based on false discovery rate (FDR) q values from DESeq2 analysis using the FactoMineR package (v2.8) [26,27].

RNA-Seq analysis for human VAT and tumors

Human colon tumor tissue and proximal VAT samples (within 1–3 cm from tumor) were collected and stored at -80°C . All shipped

samples were initially stored at -120°C and shipped on dry ice to the HCI. RNA from ~ 25 mg of tumor tissue was extracted by the Bio-repository and Molecular Pathology Shared Resource at the HCI using the Qiagen RNeasy Plus Mini Kit (Qiagen) per manufacturer's protocol. RNA from ~ 65 mg of VAT was extracted by bead beating in 1 ml TRIzol (Life Technologies) and using the PureLink RNA Mini Kit (Invitrogen) per manufacturer's protocol with 2 modifications. All centrifugation steps were performed at 4°C , and an additional centrifugation step was added before the aqueous phase separation. RNA sequencing libraries were made using the NEBNext Ultra II Directional RNA Library Prep with rRNA Depletion Kit (New England Biolabs). Sequencing was performed at the High-Throughput Genomics at the HCI on either the Illumina NovaSeq 6000 or NovaSeq X instruments using a 2×150 bp paired-end configuration at a depth of ~ 25 million reads per sample.

Raw sequencing data were converted to fastq files, and analysis was performed using a customized in-house containerized RnaAlignQC workflow. This workflow removes adapter sequences from the paired fastq reads using CutAdapt, aligns the reads to GRCh38 using the transcript-aware STAR aligner, estimates gene and isoform abundance with RNA-Seq by Expectation Maximization, assigns uniquely mapping reads with featureCounts, and calculates a variety of quality control metrics using FastQC, FastqScreen, Picard's CollectRnaSeqMetrics, and MultiQC. Low-abundance genes (<10 read counts in all samples) were removed. Differential gene expression analysis was conducted separately for tumor ($n = 193$) and VAT ($n = 198$) samples using DESeq2 (v1.42.1) [26] in R (v4.3.3). Gene counts were adjusted for relevant covariates [age (tertiles), sex (female/male), tumor stage (I/II/III), and study center (HCI/Moffitt Cancer Center/-University of Tennessee Health Science Center/Heidelberg University Hospital)] using multiple contrasts of interest based on BMI (obese compared with nonobese, obese compared with normoweight, overweight compared with normoweight, and obese compared with normoweight). A normal shrinkage estimator was applied to $\log_2\text{FC}$ estimates [26].

Gene set enrichment analysis

Gene set enrichment analysis (GSEA, v4.3.3) was performed on DESeq2 normalized data using the Molecular Signatures Database (MSigDB) Hallmark gene sets (v2024.1) [28]. Ranked gene lists were created using $\log_2\text{FC}$ values generated by DESeq2 with the normal shrinkage estimator applied, and mouse genes were collapsed and remapped to human gene symbols. Default settings were used to generate a normalized enrichment statistic for all gene sets tested. Genes contributing to the leading edge in a subset of gene sets were visualized as chord diagrams using the circlize package in R (v0.4.16) [29]. Custom gene sets were created by including all genes contributing to the leading edges of significantly enriched gene sets related to either metabolic or inflammatory processes for the identified BMI pairwise comparisons.

VAT ligand–tumor receptor analysis

Ligand–receptor analysis was conducted on paired tumor-adjacent VAT and tumor samples from 188 ColoCare patients to investigate VAT-tumor signaling. Analysis was based on normalized counts, expressed as counts per million, to assess the correlation of gene expression for each ligand–receptor pair across VAT and tumor tissues (i.e., the correlation between a ligand in VAT and its cognate receptor in tumor). The analysis was conducted separately for samples from patients across the 3 BMI categories: normoweight, overweight, and

obese. Ligand–receptor pairs were identified using 3 databases: FANTOM 5 [30], CellPhoneDB [31], and CellChat [32]. A Pearson's correlation threshold of >0.3 was set to determine significant ligand–receptor pairs, along with a requirement that at least one-third of samples from each BMI category were complete pairs (nonzero counts for both ligand and cognate receptor).

Overrepresentation analysis

Overrepresentation analysis (ORA) was performed with EnrichR [33] using 123 unique genes identified from VAT–ligand tumor–receptor pairs concordantly enriched (Pearson's correlation coefficient >0.3) in samples from patients with colon cancer and obesity.

Statistics

Statistical analyses were performed using GraphPad Prism (v10.2.2; GraphPad Software Inc). Unpaired Student's 2-tailed t tests were used to compare 2 groups. Survival analysis was conducted using log-rank (Mantel-Cox) test. $P < 0.05$ was considered statistically significant. No samples were excluded from analysis.

For RNA-Seq data, differential gene expression was determined using DESeq2 [26] at a FDR $q < 0.05$. Pairwise adonis test assessed differences in gene expression patterns between groups, where $P < 0.05$ was considered significant. Analyses of MSigDB Hallmarks using GSEA or ORA were considered significant at FDR $q < 0.05$ or adjusted $P < 0.05$, respectively.

Results

Diet-induced obesity reduces survival in an orthotopic murine organoid model of colon cancer

We determined the effect of DIO on survival in a colonoscopy-guided, organoid-based orthotopic transplantation model of murine colon cancer [22]. In this model, the engraftment rate of AKPST organoids is $>95\%$, where successful transplantation is marked by tumor formation in the submucosal layer of the colonic wall (Figure 2A, B). Review by a veterinary pathologist demonstrated that tumors are high-grade, malignant adenocarcinomas with histologic features, such as the presence of glands and the recruitment of multiple cell types, including fibroblasts and diverse immune populations, that recapitulate human colon cancer (Figure 2C, D) [22]. To generate control (nonobese) and DIO groups, mice consumed either a low-fat control diet (i.e., 10% calories from fat) or high-fat DIO diet (i.e., 60% calories from fat), respectively, ad libitum for 36–38 wk (Figure 2E), at which time DIO mice were >2 -fold heavier than Controls (Figure 2F). To assess whether DIO accelerates obesity-associated colon cancer progression, we conducted a survival study in Control and DIO mice that were orthotopically transplanted with AKPST organoids (Figure 2G). The DIO group, relative to Controls, had reduced median survival (36.5 d compared with 56.5 d; hazard ratio: 3.1; $P = 0.0002$; Figure 2H). As expected, tumors grew in all mice (Figure 2I). Furthermore, tumor mass, which was measured at the endpoint for each individual mouse, was not different between diet groups and suggests that tumor burden was the driver of the survival endpoint in both DIO and Control groups (Figure 2I). These data demonstrate that our organoid orthotopic transplantation model recapitulates key features of obesity-associated colon cancer biology.

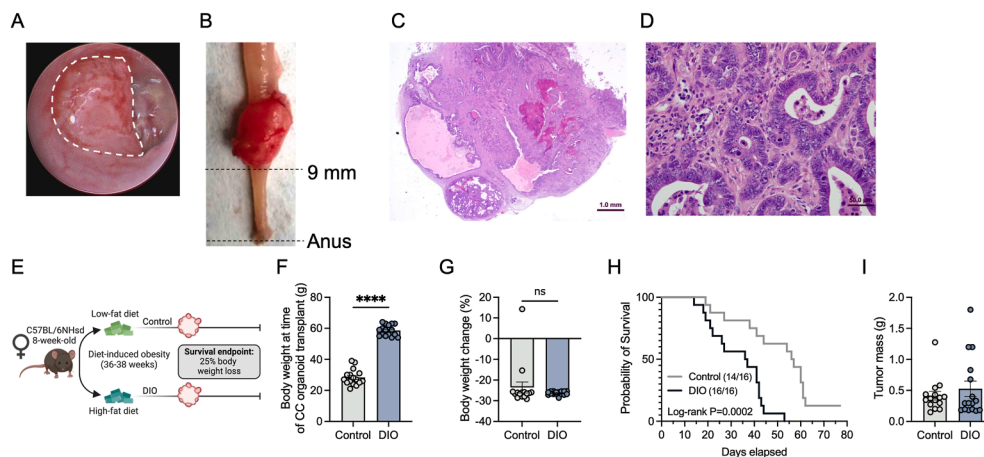


FIGURE 2. Obesity accelerates morbidity in colon tumor-bearing mice. (A) Representative colonoscopy image of a transplanted *Apc^{null} Kras^{G12D/+} Trp53^{null} Smad4^{null} tdTomato* (AKPST) organoid colon cancer tumor growing in the submucosal layer of the colonic wall. Hash marks outline the tumor. (B) Representative image of a tumor at the time of killing. Representative images of hematoxylin and eosin staining of (C) a tumor section and (D) glands, diverse immune cell types, and fibrotic material. (E) Survival study design. (F) Body weight at the time of orthotopic injection of AKPST organoids ($n = 16/\text{diet}$ treatment). (G) Percent change in body weight from day of organoid injection to killing ($n = 16/\text{diet}$ treatment). (H) Kaplan-Meier survival curve. Survival was determined as the number of days after tumor organoid injection that a tumor-bearing mouse had $\geq 25\%$ body weight loss ($n = 16/\text{diet}$ treatment). (I) Tumor mass measured at endpoint for each mouse ($n = 16/\text{diet}$ treatment). CC, colon cancer; DIO, diet-induced obesity; ns, not significant. Data are represented as mean \pm SEM (F, G, I). Statistical significance determined by unpaired 2-sided Student's *t* test (F, G, I) or Mantel-Cox test (H). **** $P < 0.0001$.

Obesity remodels metabolic and molecular profiles in an orthotopic murine organoid model of colon cancer

To identify metabolic changes potentially driving obesity-related tumor progression, we orthotopically transplanted AKPST organoids into a separate cohort of Control (nonobese) and DIO mice (after >19 wk of diet treatment) and terminated the study at an early time point (4 wk) when tumor burden was not significantly different between groups. This approach allowed for the assessment of changes associated primarily with differences in body weight phenotype rather than tumor burden (Supplemental Figure 2A). Terminal body weights and mesenteric VAT mass were increased in DIO mice relative to Control mice (Supplemental Figure 2B, C). To delineate how obesity shapes tumor cell transcriptomic profiles, we performed bulk RNA-Seq analysis on the epithelial (EpCAM⁺) tumor cell fraction from Control and DIO mice. Principal component analysis of the top 500 most differentially expressed genes in EpCAM⁺ cells revealed significant separation of the Control and DIO groups (Figure 3A). GSEA using EpCAM⁺ RNA-seq transcriptomic data and the MSigDB Hallmarks gene sets revealed stark enrichment of proliferation, metabolism, and inflammation-related gene sets in EpCAM⁺ tumor cells isolated from DIO mice relative to Control mice (Figure 3B). Within these MSigDB Hallmark gene sets, multiple genes were redundantly contained within the leading edges of these distinct pathways and processes (Figure 3C–E).

Inflammatory and metabolic pathways are concordantly enriched by obesity in human and mouse colon tumors

We next sought to determine whether the marked transcriptional changes identified in the tumors of obese mice were consistent in human colon tumors. We obtained 193 colon tumors from patients diagnosed with stage I–III colon cancer who were naïve to neo-adjuvant treatment from the ColoCare Study, a unique multisite

international prospective survivorship cohort (Table 1) [21]. Patients were on average 63 ± 13 y old, 48% female, and were classified as being normoweight (BMI <25 kg/m²; 22.7% of study population), overweight (BMI ≥ 25 – 29.9 kg/m²; 41.9% of study population), or obese (BMI ≥ 30 kg/m²; 35.5% of study population) (Table 1).

GSEA of transcriptomic profiling on patient-derived tumor samples revealed that, similar to our findings in the murine colon cancer model, a set of metabolism and inflammation pathways were uniformly enriched in tumors from patients with obesity compared with those with normal weight (Figure 4A). Moreover, within metabolism- and inflammation-related gene sets, the specific gene sets HYPOXIA, XENOBIOTIC METABOLISM, FATTY ACID METABOLISM, COMPLEMENT, ALLOGRAFT REJECTION, INFLAMMATORY RESPONSE, and IL6 JAK/STAT3 signaling were all concordantly enriched by obesity in tumors from both mice and humans. Although stratifying patient groups as obese (BMI ≥ 30 kg/m²) or normoweight (<25 kg/m²) resulted in the greatest overlap between mouse and human gene sets, there remained a consistent enrichment and substantial murine–human overlap of metabolism- and inflammation-related gene sets even when comparing obese with nonobese (BMI <30 kg/m²) or comparing overweight + obese (BMI ≥ 25 kg/m²) with normoweight (Supplemental Figure 3A, B). In contrast to findings in the murine model, proliferation processes (MYC TARGETS V2, G2M CHECKPOINT, MYC TARGETS V1, an0064 E2F TARGETS) were uniformly suppressed in tumors from patients with obesity compared with those with normal weight, indicating that obesity exerts differential, species-specific effects on proliferation pathways in the colon tumor (Figure 4A).

Given the robust overlap of specific gene sets across our murine and human analyses, we next examined whether strong enrichment scores of enriched gene sets were driven by similar genes in colon tumors from mice and humans. To do so, we created custom gene sets comprised of the leading edge genes from significantly enriched

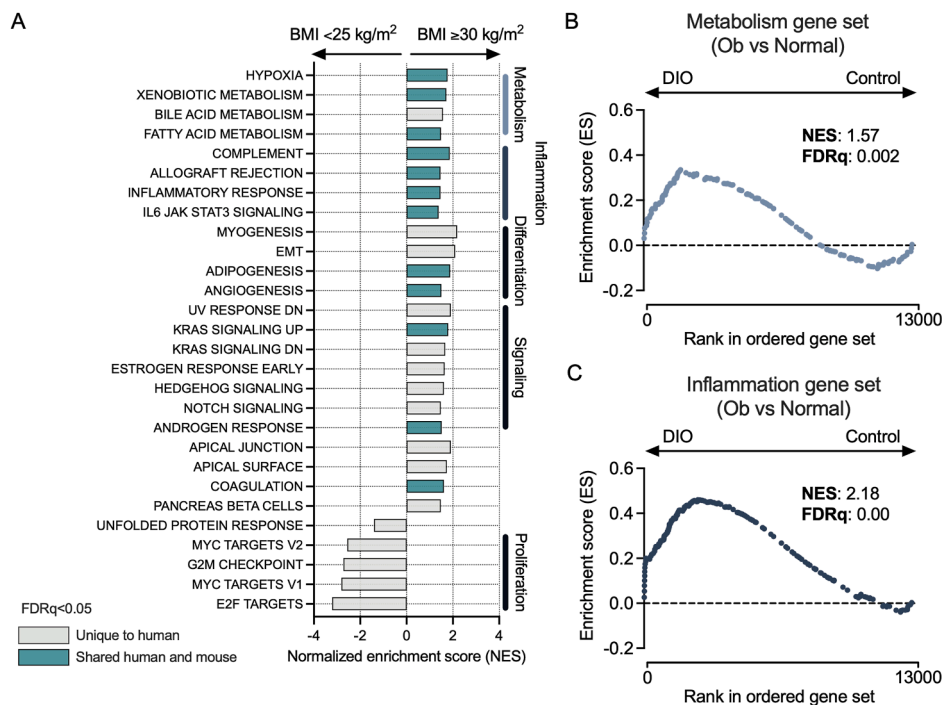


FIGURE 4. Obesity-associated enrichment of metabolic- and inflammation-related pathways is conserved between human and mouse colon tumors. (A) Gene set enrichment analysis (GSEA) using global transcriptomic data from human colon tumors. Groups were stratified by patient BMI (<25 kg/m² or ≥30 kg/m²). Hallmark gene sets commonly enriched in both human and mouse datasets are color coded in green. Hallmark gene sets unique to human colon tumors are color coded in grey. (B) GSEA enrichment plot of mouse epithelial cell adhesion molecule (EpCAM)+ colon tumor RNA-seq data using a custom metabolism gene set. This gene set was constructed from the leading-edge genes shared across all significantly enriched metabolism-related pathways identified in the human tumor dataset when stratifying patients by obese (Ob) vs. normal weight (control). (C) GSEA enrichment plot of mouse EpCAM+ colon tumor RNA-seq data using a custom inflammation gene set. This gene set was constructed from the leading edge genes shared across all significantly enriched inflammation-related pathways identified in the human tumor dataset when stratifying patients by obese vs. normal weight. Analyses of MSigDB Hallmarks using GSEA or ORA were considered significant at false discovery rate (FDR) $q < 0.05$ or adjusted $P < 0.05$, respectively. DIO, diet-induced obesity; ES, enrichment score; NES, normalized enrichment score.

genes, representing the overlap between metabolism- and inflammation-related leading edge genes in obesity-associated human and mouse colon cancer (Supplemental Table 2). Several of these genes are related to processes involved in lipid metabolism (e.g., *PLIN2*, *ACAT2*, *CD36*, and *PPDRD*), innate immune recognition (e.g., *TLR2*, *MYD88*, and *IRF4*), and tumor microenvironment modulation (e.g., *MMP9*, *MMP13*, *TGFBI*, and *SERPINE1*).

VAT-derived ligands are associated with inflammation-related pathways in obesity-driven colon cancer

To ascertain whether dysregulated VAT contributes to the obesity-associated metabolic and/or inflammatory processes observed in colon tumors through tissue-to-tissue signaling, we performed transcriptomic analyses of 188 paired human tumor-adjacent mesenteric VAT and tumor samples. To assess VAT-to-tumor signals, we utilized the FANTOM 5 [30], CellPhoneDB [31], and CellChat [32] databases to identify ligands in the VAT transcriptomic dataset and their intended receptors in the tumor transcriptomics dataset (Figure 5A). Using a correlation cutoff >0.3, we identified 88 VAT ligand–tumor receptor pairs (123 nonredundant genes) across the 3 databases that exhibited robust positive correlation only in VAT–tumor pairs from patients with BMI >30 kg/m² (Figure 5B; Supplemental Table 3). Using these 88

ligand–receptor pairs, we performed ORA with EnrichR [33] to identify significantly enriched MSigDB Hallmark 2020 gene sets (Figure 5C). As anticipated when investigating ligand–receptor interactions, many signaling-related gene sets, including UV response downregulated and Wnt β -catenin signaling, were overrepresented across the concordantly upregulated 88 VAT ligand–tumor receptor pairs (Figure 5C). Moreover, 4 inflammation-related gene sets, INFLAMMATORY RESPONSE, ALLOGRAFT REJECTION, IL6 JAK/STAT3 SIGNALING, and IL2 STAT5 SIGNALING, were consistently enriched in the ORA (using human VAT–tumor pairs) and in ≥1 GSEA (using human tumors) pairwise comparison (Figure 5D). These results indicate that obesity-associated colon cancer is associated with VAT-to-tumor signaling that enriches inflammation-related pathways.

Discussion

We report inflammation-related and, to a more modest degree, metabolic-related transcriptomic signatures as important, clinically relevant foci of VAT-to-tumor signals that we hypothesize contribute to colon cancer progression in obesity. Aberrant metabolic and inflammatory signaling are key regulators of colon cancer development

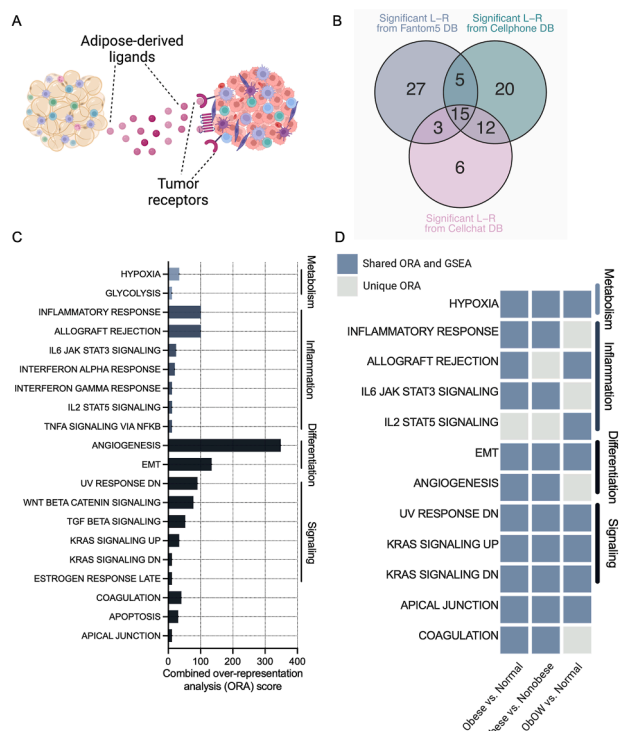


FIGURE 5. Ligand-receptor analysis reveals visceral adipose tissue (VAT)-tumor interactions that enrich for inflammation-related pathways in obesity. (A) Schematic depicting VAT-derived ligands and tumor-expressed receptors for the ligand-receptor analysis. (B) Venn diagram displaying number of ligand-receptor interactions uniquely identified in paired VAT-tumor samples from patients with obesity (BMI ≥ 30 kg/m²). Threshold for significance was defined as Pearson's correlation >0.3 between ligand and receptor expression using 3 established ligand-receptor databases: FANTOM 5, CellPhoneDB, and CellChat. (C) Overrepresentation analysis (ORA) identifying enriched Molecular Signatures Database Hallmark gene sets using the predefined list of significant ligand-receptor interactions uniquely identified in paired VAT-tumor samples from patients with obesity. (D) Heatmap indicating which Molecular Signatures Database Hallmark gene sets were concordantly enriched in both the ORA and Gene Set Enrichment Analysis (GSEA) or were unique to the ORA. Groups stratified by BMI, specifically obese vs. normal; obese vs. nonobese; or obese or overweight (ObOW) vs. normal, relate to separate GSEA analyses.

and progression [34,35]. Attempts to disentangle inflammatory and metabolic cues originating from different cell types comprising the tumor microenvironment, including infiltrating immune cells and tumor cells, are challenging [36]. Therefore, to identify changes in gene expression specific to tumor cells, we aligned the transcriptomic profiles of bulk-sequenced isolated EpCAM⁺ murine tumor cells and human colon cancer samples. We found that obesity promoted consistent enrichment of inflammation- and metabolic-related signatures across species.

There were no genes shared between human and mouse biospecimens, likely because of differences in cell composition in the respective biospecimens or variation in the expression pattern of genes across tissues, conditions, and across species [37]. To overcome this challenge, we opted to perform our analyses at the gene set level to capture the consistent estimates of biological processes or pathways between humans and mice. Indeed, when we analyzed our results at the gene set level, we observed overlapping results across species. This

higher-level approach allowed us to identify signals that may have been obscured by variations at the individual gene level.

We also established key signatures of transcriptomic remodeling of the colon tumor environment associated with obesity (BMI ≥ 30 kg/m²), which are highly consistent at even modest elevations of BMI >25 kg/m². Considerable work has attempted to understand whether and how BMI corresponds to metabolic impairment and whether alternative measures of metabolic health could be used in place of, or in addition to, BMI to appropriately stratify risk for chronic diseases such as colon cancer [38–41]. Our key findings were robust across 3 distinct BMI thresholds (obese compared with normoweight, obese + overweight compared with normoweight, and obese compared with nonobese), each of which were associated with the inflammatory and metabolic tumoral signaling networks that we identified.

Using transcriptomic profiling, we identified that the inflammation-related leading edge genes overlapping between murine and human transcriptomic datasets were related to innate immune sensing (*TLR2*, *MYD88*, and *IRF4*) and tumor microenvironment remodeling (*MMP9*, *TGFBI*, and *SERPINE1*). Production of immunomodulatory proteins such as TGF β and SERPINE1, which we found to be associated with obesity across mouse and human colon cancer, engenders an immunosuppressive tumor microenvironment and thereby promotes colon cancer progression [42–45]. TGF β production in colon tumors strongly predicts disease progression, and inhibition of TGF β signaling not only restores tumor immunosurveillance but also limits tumor progression [42,43]. Importantly, such restoration of immunosurveillance is potentially synergistic with immune checkpoint inhibition [43]. SERPINE1, like TGF β , is abundantly expressed by colon tumors [44], and enhances colon tumor growth through blunting of antitumor immunity, specifically by promoting the exclusion of cytotoxic CD8⁺ T cells from the tumor microenvironment [45]. Obesity promotes TGF β and SERPINE1 expression in contexts outside of colon cancer [46,47]; thus, this signaling axis may be translationally actionable in limiting obesity-driven colon cancer.

In regard to the obesity-related changes in immune-sensing genes, toll-like receptor (TLR) proteins sense pathogen- or damage-associated molecular patterns to induce innate immune signaling responses and play important roles in the development of various inflammatory and metabolic diseases [48]. In humans and murine models, these pathways are critical for the maintenance of effective intestinal barrier function and physical separation from the intestinal microbiota [49]. MYD88, an adaptor protein critical for a range of TLR signaling, is required for intestinal barrier function [49]. Epithelial cell intrinsic TLR2-MYD88 signaling supports the proliferation of tumor cells and enables carcinogenesis [50]. Similarly, MYD88-dependent TLR4 signaling supports the acceleration of colon cancer progression by high-fat diet feeding [51]. High-fat diets themselves remodel the intestinal microbiome and promote the presence of microbes that produce metabolites that can impair intestinal barrier function and thereby promote colon cancer carcinogenesis [52]. By identifying, for example, TLR2-MYD88 signaling as a conserved feature of obesity-induced inflammation in colon cancer, this work bridges critical mechanistic findings on the role of inflammation in cancer progression with the modifiable risk factor of obesity.

In addition to synthesizing preclinical and clinical findings by integrating transcriptomic signatures across 2 species, another strength of our study was the assessment of putative interactions between VAT-derived factors and tumor signaling in clinical samples. It is well established that VAT expands and becomes dysregulated in obesity and is a poor prognostic factor for colon cancer, highlighting the potential

role of adipose-derived cytokines, adipokines, lipid mediators, and other factors to promote the incidence and progression of colon cancer [8,9,11]. Specifically in colon cancer, mesenteric VAT envelopes the colonic serosa, and our clinical team collected VAT samples within 1 to 3 cm of the tumor to ensure anatomical proximity to the cancer. Using 3 established databases to build an extensive list of ligand–receptor pairs, we found that correlated expression (Pearson's coefficient >0.3) of cognate pairs of ligands from VAT and receptors in tumors from patients with obesity were overrepresented across 7 inflammation-related gene sets (INFLAMMATORY RESPONSE, ALLOGRAFT REJECTION, IL6 JAK STAT3 SIGNALING, INTERFERON ALPHA RESPONSE, INTERFERON GAMMA RESPONSE, IL2 STAT5 SIGNALING, and TNFA SIGNALING VIA NFKB). Thus, specifically in patients with obesity, signals from mesenteric VAT may be a source driving inflammatory responses in colon tumors, and further work elucidating these signaling networks may be tractable in orthotopic murine models.

Obesity remodels metabolic programs of various tissues at both cellular and systemic levels, and our aligned preclinical and clinical datasets identify metabolic alterations that are conserved between obesity-driven colon cancer in mice and humans. Specifically, we found numerous lipid metabolism genes to be induced by obesity in colon cancer (e.g., *PLIN2*, *ACAT2*, *CD36*, and *PPARD*). Colon tumors enhance the expression of lipogenic enzymes to sustain considerable enrichment of triacylglycerol relative to normal tissue [53]. Yet, the roles of dietary lipid supply and obesity in supporting such metabolic profiles are poorly understood. Our finding that enrichment of lipid metabolism–related genes is a feature of obesity-driven transcriptomic remodeling in colon cancer cells demonstrates that while lipid metabolism is reprogrammed by tumor cell–intrinsic processes, colon cancer cells remain sensitive to the systemic metabolic state.

There were limitations of our analyses. First, we were unable to determine whether alternative measures of metabolic disease or increased adiposity similarly remodel colon cancer transcriptomic programs. Indeed, colon tumors from patients with type 1 diabetes compared with type 2 diabetes demonstrate distinct gene expression profiles [41]. We have also previously shown that the amount of VAT is correlated with distinct transcriptomic patterns in VAT [9]. Thus, our results indicate that although stratification by BMI is sufficient to identify colon tumor remodeling, contributions from other factors, such as the amount of visceral fat and/or specific metabolic perturbations, are possible. As an additional limitation, we did not evaluate ethnicity or other social determinants of health in a comprehensive framework [54]. In addition, BMI has limitations as an estimator of body composition, although we observed cross-species concordance in obesity-associated biological pathways, increasing confidence in these novel findings. Moreover, this is the first large study using colon tumor and adjacent adipose tissue biospecimens surgically collected across 4 sites spanning the United States and Germany. Therefore, our results are both internally valid and generalizable across several populations.

In summary, integrating complementary data from well-controlled mouse studies and clinical samples from a representative sample of patients with newly diagnosed colon cancer, we identified obesity-responsive, cancer-promoting pathways and genes common to mice and humans. These findings, although informative, cannot replace the need for definitive mechanistic studies to define the extent to which these pathways direct colon cancer progression. In our murine organoid model of colon cancer, obesity reduced survival and enriched several MSigDB Hallmark gene sets involving inflammation, metabolism, and proliferation. In patients with colon cancer,

obesity-enriched inflammation and metabolic pathways in concordance with our mouse model findings. Moreover, human transcriptomic analyses uncovered procancer interactions between VAT-derived ligands and tumor-intrinsic receptors. Synthesis of our murine and human findings enables prioritization of new molecular targets underlying the obesity–colon cancer association and provides a strong foundation for future translational studies to develop and test mechanism-based lifestyle or pharmacologic interventions.

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Author contributions

The authors' responsibilities were as follows – EMG, TL, VMB, ACT, JR, CMU, SDH: designed research; EMG, TL, BM, SSK, BG, CAW, OA, MFC, AC, CB, PS, IS, SH, JNC, JJ, AB: conducted research; VMB, CAW, CH, JO, CMU: administered project; BG, MAS, CK, EMS, DAB, ATT, DS, CIL, JCF, ACT, JR, CMU, SDH: provided essential materials; EMG, TL, MFC, DAN, KB: analyzed data; EMG, SDH: wrote paper; and all authors: read and approved the final manuscript.

Conflict of interest

CMU has as cancer center director oversight research funded by several pharmaceutical companies but has not received funding directly herself. All other authors report no conflicts of interest.

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Data availability

Murine transcriptomic data have been deposited to GEO with accession number GSE284066 and will be publicly available as of the date of publication. ColoCare Study data are available from colocarestudy_admin@hci.utah.edu on reasonable request and as

described on the ColoCare website (<https://uofuhealth.utah.edu/huntsman/labs/colocare-consortium/>). Our data sharing procedures are available online (<https://uofuhealth.utah.edu/huntsman/labs/colocare-consortium/data-sharing/new-projects.php>). For any additional questions, please contact the ColoCare Study Administrator Team (colocarestudy_admin@hci.utah.edu). Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Stephen D. Hursting (hursting@email.unc.edu).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ajcnut.2025.09.031>.

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