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Gastrointestinal Symptom Cluster is Associated With Epigenetic Regulation of Lymphotoxin Beta in Oncology Patients Receiving Chemotherapy

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Abstract

Objectives: While the gastrointestinal symptom cluster (GISC) is common in patients receiving chemotherapy, limited information is available on its underlying mechanism(s). Emerging evidence suggests a role for inflammatory processes through the actions of the nuclear factor kappa B (NF-κB) signaling pathway. This study evaluated for associations between a GISC and levels of DNA methylation for genes within this pathway.

Methods: Prior to their second or third cycle of chemotherapy, 1071 outpatients reported symptom occurrence using the Memorial Symptom Assessment Scale. A GISC was identified using exploratory factor analysis. Differential methylation analyses were performed in two independent samples using EPIC (n = 925) and 450K (n = 146) microarrays. Trans expression-associated CpG (eCpG) loci for 56 NF-κB signaling pathway genes were evaluated. Loci significance were assessed using an exploratory false discovery rate (FDR) of 25% for the EPIC sample. For the validation assessment using the 450K sample, significance was assessed at an unadjusted p-value of 0.05.

Results: For the EPIC sample, the GISC was associated with increased expression of lymphotoxin beta (*LTB*) at one differentially methylated trans eCpG locus (cg03171795; FDR = 0.168). This association was not validated in the 450K sample.

Conclusions: This study is the first to identify an association between a GISC and epigenetic regulation of a gene that is involved in the initiation of gastrointestinal immune responses. Findings suggest that increased *LTB* expression by hypermethylation of a trans eCpG locus is involved in the occurrence of this cluster in patients receiving chemotherapy. LTB may be a potential therapeutic target for this common cluster.

Keywords
cancer, chemotherapy, DNA methylation, gastrointestinal symptom cluster, inflammation, nausea

Introduction

A gastrointestinal symptom cluster is one of the most common clusters in patients receiving chemotherapy (Harris, Kober, Conley, et al., 2022; Sullivan et al., 2018). While this cluster is stable across dimensions of the symptom experience regardless of cancer types (Harris, Kober, Cooper, et al., 2022), the consistency of the symptoms within this cluster is variable across dimensions and time (Molassiotis et al., 2010; Skerman et al., 2012). These findings are not surprising given the relatively high occurrence rates for individual gastrointestinal symptoms. For example, in a heterogenous sample of oncology patients (Harris, Kober, Cooper, et al., 2022), 49.4% reported change in the way food tastes; 47.5% reported nausea; and 43.5% reported constipation prior to the start of their second or third cycle of chemotherapy. In addition,

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patients identified these symptoms were some of the most severe and distressing. Of note, the gastrointestinal symptom cluster (referred to as gastrointestinal cluster in the remainder of the manuscript) is associated with lower functional status (Chen & Lin, 2007; Suwisith et al., 2008) and poorer quality of life (Matzka et al., 2018; Pirri et al., 2013; Ren et al., 2017). In addition, poor management of the symptoms within this cluster are associated with increased economic burden (Carlotto et al., 2013).

While no study has investigated the underlying mechanism(s) for a gastrointestinal cluster, a growing body of evidence suggests that inflammatory mechanisms play a role in the development of gastrointestinal symptoms in oncology patients (Cinausero et al., 2017). Following the administration of chemotherapy, a cascade of biological processes is triggered that result in mucosal inflammation of the entire alimentary tract (Sonis et al., 2004). In the first stage, gastrointestinal mucositis is initiated by increases in oxidative stress; production of reactive oxygen species (ROS); deoxyribonucleic acid (DNA) damage; and activation of innate immunity. Next, ROS and the innate immune system accelerate various inflammatory responses through macrophage stimulation and transcription factor activation. Both of these processes lead to the production of various proinflammatory cytokines (e.g., interleukin (IL) 6, tumor necrosis factor (TNF) α) which in turn activate multiple signaling pathways (e.g., mitogen-activated protein kinase, nuclear factor kappa B (NF-κB)). Once activated, NF-κB signaling induces the production of additional proinflammatory cytokines (Oeckinghaus & Ghosh, 2009). Ultimately, these processes culminate in tissue ulceration. Of note, mucositis of the gastrointestinal tract is associated with multiple symptoms, including abdominal bloating, constipation, diarrhea, mouth sores, nausea, vomiting, and pain (Gibson & Keefe, 2006).

Of the various transcription factors that are activated as part of this inflammatory cascade, NF-κB is hypothesized to play a central role (Sonis, 2002). Three studies have evaluated for associations between mucositis or a gastrointestinal symptom and differences in NF-κB signaling. In the first study that evaluated for differential expression in the stomach, jejunum, and colon of rats treated with irinotecan (Bowen et al., 2007), multiple genes within the NF-κB signaling pathway were upregulated. In another study of patients undergoing chemoradiation (Sonis et al., 2007), more severe mucositis was associated with perturbations in the NF-κB signaling pathway. In a third study that compared patients with and without chemotherapy-induced nausea (Singh, Dhruva, et al., 2020), perturbations were identified in a number of inflammatory pathways, including the NF-κB signaling pathway.

While these findings support the hypothesis that NF-κB signaling is involved in the development of a variety of symptoms associated with chemotherapy-induced injury of the gastrointestinal mucosa, the specific processes involved in its regulation warrant additional research. One approach is to evaluate epigenetic regulation of the genes in this pathway. While DNA methylation allows the body to adapt to internal and external stimuli, dysregulation of epigenetic processes may influence the development or severity of symptoms. For example, in women with breast cancer whose cognitive function was assessed prior to and following the receipt of chemotherapy (Yang et al., 2020), lack of improvement in the memory domain, at 1 year after the initiation of chemotherapy, was associated with 56 differentially methylated loci.

While previous research has focused primarily on promoter associated epigenetic regulation of gene expression (Jones, 2012), emerging evidence suggests that methylation of a CpG locus on one chromosome can regulate the transcription of a gene on another chromosome (i.e., trans CpG; Kennedy et al., 2018; Portela & Esteller, 2010). For example, methylation of trans CpGs can influence gene expression (i.e., trans expression-associated CpG (eCpG) by binding to enhancer elements or transcription factor binding sites (Kennedy et al., 2018). Given the preliminary evidence of associations between chemotherapy-induced gastrointestinal symptoms and NF-κB, this analysis evaluated for associations between the occurrence of a gastrointestinal cluster and levels of DNA methylation on trans eCpG loci for genes within the NF-κB pathway.

Methods

Patients and Settings

This analysis is part of a larger study that evaluated symptom clusters in oncology outpatients receiving chemotherapy (Harris, Kober, Cooper, et al., 2022). Eligible patients were ≥18 years of age; had a diagnosis of breast, lung, gastrointestinal, or gynecologic cancer; had received chemotherapy within the preceding 4 weeks; were scheduled to receive at least two additional cycles of chemotherapy; were able to read, write, and understand English; and gave written informed consent. Patients were recruited from two Comprehensive Cancer Centers, one Veteran's Affairs hospital, and four community-based oncology programs.

Study Procedures

The study was approved by the Institutional Review Board at each of the study sites. Of the 2234 patients approached, 1343 consented to participate (60.1% response rate). The major reason for refusal was being overwhelmed with their cancer treatment. Eligible patients were approached in the infusion unit during their first or second cycle of chemotherapy by a member of the research team to discuss study participation and obtain written informed consent. Data from the enrollment assessment (i.e., symptoms in the week prior to the patient's second or third cycle of chemotherapy) were

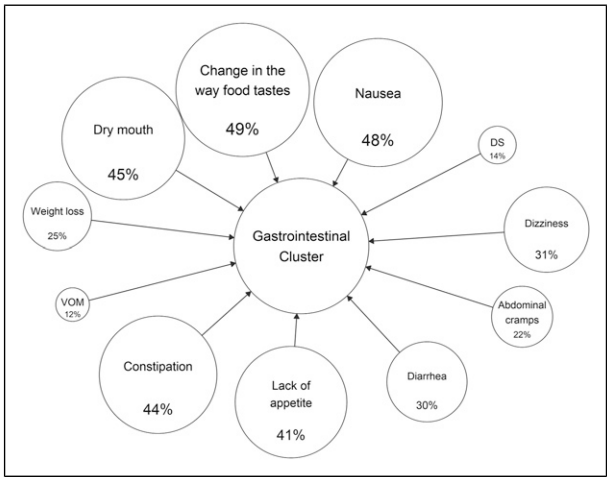

Figure 1. Symptoms within the gastrointestinal symptom cluster. The size of each node represents the occurrence rate for that symptom in oncology patients in the week prior to their second or third cycle of chemotherapy (Harris, Kober, Cooper, et al., 2022). *Note*. DS = difficulty swallowing; VOM = vomiting.

used in this analysis. At enrollment, a total of 1071 patients provided a blood sample for the DNA methylation analyses. Medical records were reviewed for disease and treatment information.

Instruments

Patients completed a demographic questionnaire, Karnofsky Performance Status (KPS) scale (Karnofsky, 1977), and Self-Administered Comorbidity Questionnaire (Sangha et al., 2003). Toxicity of each patient's chemotherapy regimen was rated using the MAX2 index (Extermann et al., 2004).

A modified version of the 32-item Memorial Symptom Assessment Scale (MSAS) was used to evaluate the occurrence, severity, and distress of 38 common symptoms associated with cancer and its treatment (Portenoy et al., 1994). Six additional symptoms were added: hot flashes, chest tightness, difficulty breathing, abdominal cramps, increased appetite, and weight gain. Using the MSAS, patients were asked to indicate whether they had experienced each symptom in the past week (i.e., symptom occurrence). The patients' responses to the occurrence items were used to create the symptom clusters. The validity and reliability of the MSAS are well-established (Portenoy et al., 1994).

Data Analyses

Descriptive statistics and frequency distributions were calculated for the demographic and clinical characteristics, using the Statistical Package for the Social Sciences Version 27 (IBM Corporation, Armonk, NY). Exploratory factor analysis (EFA) was used to identify symptom clusters using Mplus Version 8.6 (Muthén & Muthén, 2019).

Methods for the EFA were reported elsewhere (Harris, Kober, Cooper, et al., 2022). In brief, using the dichotomous occurrence items, tetrachoric correlations were used to create the matrix of associations (Muthén & Muthén, 2019). The simple structure for the occurrence EFA was estimated using the method of unweighted least squares with geomin (i.e., oblique) rotation (Muthén & Muthén, 2019). Factor loadings were considered meaningful if the loading was ≥0.40 (Muthén & Muthén, 2019). Factors (i.e., symptom clusters) were adequately defined if at least two items (i.e., symptoms) had loadings of ≥0.40 (Brown, 2015). Clusters were named based on the symptoms with the highest factor loadings and the majority of the symptoms within the cluster.

With these methods, a gastrointestinal cluster (Figure 1) was identified in our previous analysis (Harris, Kober, Cooper, et al., 2022). A factor score was calculated as the sum of the occurrence ratings for the 11 symptoms within the cluster (range of 0–11). Initially, the DNA methylation analyses were conducted using the patients' symptom cluster factor scores as a continuous value. However, the *p*-value distribution for the differential methylation tests across the genome was severely conservative (i.e., underabundance of low *p*-values; data not shown). Therefore, for the current analyses, the total factor score was dichotomized into two groups (i.e., 0 symptoms = no gastrointestinal cluster group vs. 1–11 symptoms = gastrointestinal cluster group).

Selection of Trans eCpG Loci

Candidate genes in the NF-κB signaling pathway were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa & Goto, 2000; Supplemental Figure 1). Then, CpG sites for these genes that were known to have methylation values associated with changes in gene expression on another chromosome (i.e., trans eCpGs; Kennedy et al., 2018) were selected. These trans eCpG loci for genes within the NF-κB signaling pathway were evaluated for associations with gastrointestinal cluster group membership.

Biospecimen Processing, Quantification of Methylation Status, and Quality Control

Methods for the DNA methylation analyses are described in detail elsewhere (Kober et al., 2020). In brief, DNA was extracted from archived buffy coats using the PUREGene DNA isolation kit (Invitrogen, Carlsbad, CA); quantified using a NanoDrop UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA); and normalized to a concentration of 50 ng per microliter. DNA was bisulfite converted using the Zymo EZ-96 DNA Methylation Kit (Catalog #D5004) Deep-Well Format (Zymo Research, Irvine, CA) and used as input

for the Infinium HD Methylation Assay (Illumina Inc, San Diego, CA).

Of the 1071 patients in this study, DNA methylation was measured for 925 patients using the Infinium MethylationEPIC BeadChip (i.e., EPIC microarray sample) and for 146 patients using the Infinium HumanMethylation 450 BeadChip (i.e., 450K microarray sample; Illumina, Inc, San Diego, CA). The EPIC microarray sample was used as a discovery sample while the 450K microarray sample was used as a validation sample. All of the samples were scanned on the Illumina iScan (Illumina, Inc, San Diego, CA). Preliminary analysis and quality control procedures were performed using GenomeStudio (Illumina, Inc, San Diego, CA). Samples that had <90% of their targets detected at a p-value of ≤ 0.01 were flagged for review. Sample replicates and Jurkat control replicates were checked to ensure an r^2 value of > 0.99.

Subsequent analyses were done using well-established protocols in R (version 4.1.0; Bock, 2012). Corrections for Infinium I and II probes, balance correction, background correction, and quantile normalization were performed using the minfi package in R (version 1.38.0; Aryee et al., 2014; Du et al., 2008). Probes that contained a single nucleotide polymorphism at a CpG or flanking site and probes that aligned with multiple places on the genome were excluded (Chen et al., 2013). Methylation scores were quantified as M-values (Du et al., 2010).

DNA Methylation Analyses

Given that DNA methylation levels differ among blood cell types (McGregor et al., 2016), cell types were estimated using the *estimateCellCounts2()* function in the FlowSorted.Blood. EPIC R package (version 1.10.1; Salas & Koestler, 2021). Cell type deconvolution was performed using the IDOL L-DMR library for cluster of differentiation 8 (CD8) and CD4 T-cells, natural killer cells, B cells, monocytes, and neutrophils (Salas et al., 2018). Differences in estimates of cell type composition between the gastrointestinal cluster groups were evaluated using Welch two sample t-tests and assessed for significance at a p-value of <0.05. Any cell type composition estimates that were significantly associated with membership in the gastrointestinal cluster group were included as covariates in the final model. Given that methylation status changes over the lifespan (Jones et al., 2015), age was included as a covariate in the final regression model. Surrogate variable analysis, using the Leek method (R package version 3.4.0; Leek & Storey, 2007), was used to estimate surrogate variables for technical and non-technical variations that contributed to heterogeneity in the sample that were not due to the gastrointestinal cluster group, age, or cell type.

To evaluate for associations between gastrointestinal cluster group membership and methylation status of trans eCpG loci for NF-κB genes, tests for differentially methylated probes (DMPs) were done using a generalized linear model implemented in the limma R package using the "ls" method (version 3.48.3; Ritchie et al., 2015). The significance of the DMPs for each of the NF-κB genes was assessed using an exploratory false discovery rate (FDR) of 25% under the Benjamini-Hochberg procedure for the EPIC microarray sample (Benjamini & Hochberg, 1995). Then, candidate trans eCpG loci identified as differentially methylated in the EPIC microarray sample were evaluated for differential methylation in the 450K microarray sample (Supplemental Figure 1). For the validation assessment using the 450K microarray sample, significance of the candidate trans eCpG loci was assessed at an unadjusted p-value of 0.05.

Finally, in order to characterize potential functional roles for these eCpGs, we evaluated for evidence of regulatory elements in regions surrounding these loci using annotation data from the Encyclopedia of DNA Elements (ENCODE; Rosenbloom et al., 2013) obtained from the University of California Santa Cruz Genome Browser (Kent et al., 2002). Finally, we identified predicted functional partners for the genes with differentially methylated trans eCpGs from a protein-protein interaction network that was created using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (Szklarczyk et al., 2019).

Results

Demographic and Clinical Characteristics

Of the 925 patients in the EPIC microarray sample, one was excluded for insufficient phenotypic data and one for poor sample quantification. Of the remaining 923 patients, 76.2% were female, 69.4% were White, 64.1% were married or partnered, and had a mean age of 57.5 (±12.2) years (Table 1). Most patients were well-educated (16.1 ± 3.0 years), exercised on a regular basis (71.6%), and had never smoked (66.4%). Patients had an average of 2.4 (±1.4) comorbid conditions and a KPS score of 80.4 (±12.6). The most common type of cancer was breast (39.5%), followed by gastrointestinal (34.0%), gynecological (15.9%), and lung (10.5%). Patients reported 13.5 (±7.1) concurrent symptoms before their second or third cycle of chemotherapy.

Of the 146 patients in the 450K microarray sample, 100% were female, 65.5% were White, 67.6% were married or partnered, and had a mean age of 52.7 (±11.7) years (Table 2). Most patients were well-educated (16.3 ± 2.9 years), exercised on a regular basis (75.7%), and had never smoked (72.4%). Patients had an average of 2.4 (±1.4) comorbid conditions and a KPS score of 79.1 (±11.6). The most common type of cancer was breast (99.3%) followed by gastrointestinal (0.7%). Patients reported 16.0 (±7.8) concurrent symptoms before their second or third cycle of chemotherapy.

DNA Methylation Analyses

For the EPIC microarray sample, of the 90 genes that were identified in the NF-κB signaling pathway, 3785 trans eCpG

Table 1. Demographic and Clinical Characteristics of the Patients in the EPIC Microarray Sample (n = 923).

Characteristic	Mean		*SD*
Age (years)	57.5		12.2
Education (years)	16.1		3.0
Body mass index (kilograms per metered squared)	26.1		5.6
Karnofsky performance status score	80.4		12.6
Number of comorbidities out of 13	2.4		1.4
Self-administered comorbidity questionnaire score	5.4		3.2
Time since cancer diagnosis (years)	1.9		3.9
Time since diagnosis (median)		0.42	
Number of prior cancer treatments (out of 9)	1.5		1.5
Number of metastatic sites including lymph node involvement (out of 9)	1.2		1.2
Number of metastatic sites excluding lymph node involvement (out of 8)	0.8		1.0
MAX2 index of chemotherapy toxicity score (0–1)	0.17		0.08
Mean number of MSAS symptoms (out of 38)	13.5		7.1
Characteristic	***n***		**(%)**
Gender			
Female	703		76.2
Male	220		23.8
Ethnicity			
Asian or pacific islander	114		12.4
Black	71		7.8
Hispanic, mixed, or other	95		10.4
White	636		69.4
Married or partnered			
No	326		35.9
Yes	581		64.1
Lives alone			
No	713		78.4
Yes	196		21.6
Child care responsibilities			
No	709		79.0
Yes	188		21.0
Care of adult responsibilities			
No	767		92.6
Yes	61		7.4
Currently employed			
No	585		64.1
Yes	327		35.9
Income			
<US$30,000	142		17.3
US$30,000 to <US$70,000	172		20.9
US$70,000 to <US$100,000	143		17.4
≥US$100,000	365		44.4
Exercise on a regular basis			
No	255		28.4
Yes	643		71.6
Current or history of smoking			
No	603		66.4
Yes	305		33.6
Type of cancer			
Breast	365		39.5
Gastrointestinal	314		34.0

(continued)

Table 1. (continued)

Characteristic	*n*	(%)
Gynecological	147	15.9
Lung	97	10.5
Type of prior cancer treatment		
No prior treatment	238	26.7
Only CTX, surgery, or RT	375	42.0
CTX and surgery, or CTX and RT, or surgery and RT	175	19.6
CTX and surgery and RT	104	11.7
Cycle length		
14 days	417	45.3
21 days	438	47.6
28 days	65	7.1
Emetogenicity of the chemotherapy regimen		
Minimal/low	161	17.5
Moderate	580	63.0
High	180	19.5
Antiemetic regimen		
None	56	6.2
Steroid alone or serotonin receptor antagonist alone	185	20.4
Serotonin receptor antagonist and steroid	436	48.2
NK-1 receptor antagonist and two other antiemetics	228	25.2

Note. CTX = chemotherapy, MSAS = Memorial Symptom Assessment Scale, NK-1 = neurokinin 1, RT = radiation therapy, SD = standard deviation.

loci across 56 genes were evaluated for differential methylation. Because cell type composition was not associated with gastrointestinal cluster group membership, only age and 22 surrogate variables were included as covariates in the final model. For the 450K microarray validation sample, because cell type composition was not associated with gastrointestinal cluster group membership, only age and one surrogate variable were included as covariates in the final model.

For the EPIC microarray sample, hypermethylation of one trans eCpG locus (i.e., cg03171795) for the lymphotoxin beta (*LTB*) gene was found to be significantly associated with the occurrence of the gastrointestinal cluster (FDR = 0.168). For the 450K microarray sample, while no association was found ($p = 0.664$), the direction of methylation for this locus was the same across both samples (i.e., positive log fold change; view supplemental data files at https://zenodo.org/record/6638570).

Discussion

This exploratory study is the first to evaluate for an association between a gastrointestinal symptom cluster and a specific inflammatory mechanism; namely epigenetic regulation of the NF-κB pathway. This cluster was associated with hypermethylation of one trans eCpG locus (i.e., cg03171795). While located on chromosome 3, this trans eCpG locus regulates the expression of *LTB* which is a gene located within the major histocompatibility complex on chromosome 6. In another study (Kennedy et al., 2018), hypermethylation of this eCpG locus was associated with increased expression of *LTB*. Notably, LTB is situated at the beginning of the NF-κB signaling pathway and can induce NF-κB signaling. This finding supports previous research that suggests that signaling within the NF-κB pathway is involved in chemotherapy-induced inflammation along the entire gastrointestinal tract (Bowen et al., 2007; Singh, Dhruva, et al., 2020; Sonis et al., 2007).

Regulatory Role of Trans eCpG Locus

Given that epigenetic modifications are dynamic and multiple factors influence gene expression, one of the challenges for methylation association studies is the establishment of a functional role for the epigenetic variation that is identified (Rakyan et al., 2011). As illustrated in Figure 2, multiple sources of independent and complementary data show that the trans eCpG locus, cg03171795, is located within a putative regulatory region of chromosome 3. Specifically, evidence of regulatory elements compiled by ENCODE suggests that this locus is situated within a region of enhancer activity (ENCODE Project Consortium, 2012; Ernst et al., 2011). Enhancer regions are areas of non-coding DNA that enhance gene transcription through the recruitment of transcription factors and RNA polymerase II and modification of chromatin accessibility (Karnuta & Scacheri, 2018). Located distal to their target genes, enhancers form loops to move in closer to the promoter region of a gene (Andersson et al., 2015). Our

Table 2. Demographic and Clinical Characteristics of the Patients in the 450K Microarray Sample (n = 146).

Characteristic	Mean		*SD*
Age (years)	52.7		11.7
Education (years)	16.3		2.9
Body mass index (kilograms per meters squared)	26.3		6.4
Karnofsky performance status score	79.1		11.6
Number of comorbidities out of 13	2.4		1.4
Self-administered comorbidity questionnaire score	5.5		3.1
Time since cancer diagnosis (years)	3.0		4.7
Time since diagnosis (median)		0.43	
Number of prior cancer treatments (out of 9)	2.0		1.9
Number of metastatic sites including lymph node involvement (out of 9)	1.0		1.3
Number of metastatic sites excluding lymph node involvement (out of 8)	0.6		1.1
MAX2 index of chemotherapy toxicity score (0–1)	0.20		0.09
Mean number of MSAS symptoms (out of 38)	16.0		7.8
Characteristic	***n***		**(%)**
Gender			
Female	146		100.0
Ethnicity			
Asian or pacific islander	24		16.6
Black	10		6.9
Hispanic, mixed, or other	16		11.0
White	95		65.5
Married or partnered			
No	47		32.4
Yes	98		67.6
Lives alone			
No	120		82.8
Yes	25		17.2
Child care responsibilities			
No	100		69.0
Yes	45		31.0
Care of adult responsibilities			
No	120		89.6
Yes	14		10.4
Currently employed			
No	96		66.2
Yes	49		33.8
Income			
< $30,000	32		24.4
$30,000 to < $70,000	22		16.8
$70,000 to < $100,000	19		14.5
≥ $100,000	58		44.3
Exercise on a regular basis			
No	35		24.3
Yes	109		75.7
Current or history of smoking			
No	105		72.4
Yes	40		27.6
Type of cancer			
Breast	145		99.3
Gastrointestinal	1		0.7

(continued)

Table 2. (continued)

Characteristic	*n*	(%)
Type of prior cancer treatment		
No prior treatment	34	23.3
Only CTX, surgery, or RT	62	42.5
CTX and surgery, or CTX and RT, or surgery and RT	18	12.3
CTX and surgery and RT	32	21.9
Cycle length		
14 days	48	32.9
21 days	86	58.9
28 days	12	8.2
Emetogenicity of the chemotherapy regimen		
Minimal/low	43	29.5
Moderate	57	39.0
High	46	31.5
Antiemetic regimen		
None	21	15.1
Steroid alone or serotonin receptor antagonist alone	30	21.6
Serotonin receptor antagonist and steroid	51	36.7
NK-1 receptor antagonist and two other antiemetics	37	26.6

Note. CTX = chemotherapy; MSAS = Memorial Symptom Assessment Scale; NK-1 = neurokinin one; RT = radiation therapy; SD = standard deviation.

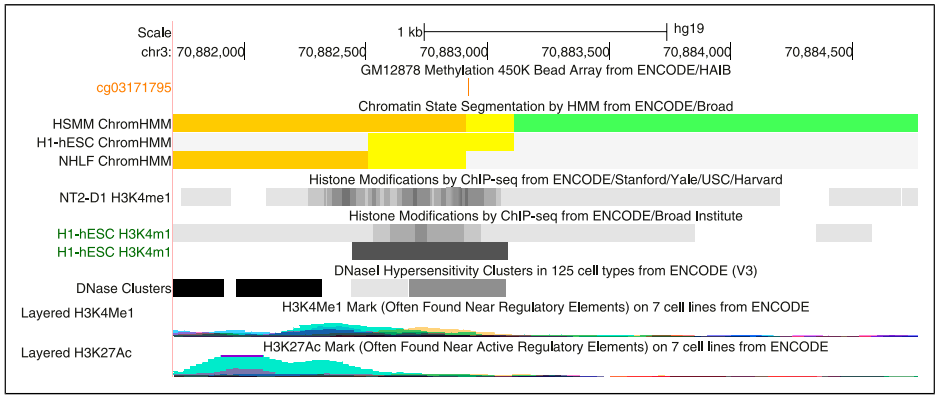

Figure 2. Screenshot of the University of California Santa Cruz Genome browser (http://genome.ucsc.edu/) displaying cg03171795 on chromosome three of the hg19 (genome reference consortium Version 37) assembly of the human genome (Kent et al., 2002). Assembly tracks show scale, chromosome, and the hypermethylated status of cg03171795 and its genomic position as reported by the HAIB. Tracks denoting putative regulatory regions across multiple cell lines that were identified by ENCODE include: predicted chromatin state using an HMM; histone modifications for H3K4me1 and H3K4m1; DNase I hypersensitivity clusters; and levels of enrichment for the layered H3K4Me1 and H3K27Ac histone marks. For the three tracks that illustrate the ChromHMM for three cell lines, the orange color indicates a "strong enhancer" predicted chromatin state; yellow color indicates a "weak/poised enhancer" state; and light grey color indicates heterochromatin or low signal. For the H3K4Me1 and H3K27Ac marks, the coloring indicates the signal intensity from one of seven cell lines.
Note. ChromHMM = chromatin state segmentation by multivariate Hidden Markov Modeling; ENCODE = Encyclopedia of DNA elements; GM12878 = B-lymphoblastoid cell line; H1-hESC = embryonic stem cells, line H1; H3K4me1 = histone H3 mono methyl K4; HAIB = Hudson Alpha Institute for Biotechnology; hg = human genome; HMM = Hidden Markov Model; HSMM = human skeletal muscle myoblasts; NT2-D1 = clonally derived, pluripotent human embryonal carcinoma cell line.

findings are consistent with a study that sought to identify and characterize genome-wide eCpGs in two independent datasets and found that eCpGs were enriched for enhancer annotations and transcription factor binding sites, particularly among trans eCpGs (Kennedy et al., 2018).

Role of LTB in Inflammatory Processes

LTB is a member of the TNF super family of ligands. Along with LTA, it forms a heterotrimer complex (i.e., LTA_1B_2) that exclusively binds to the LTB receptor (LTBR; Borelli & Irla,

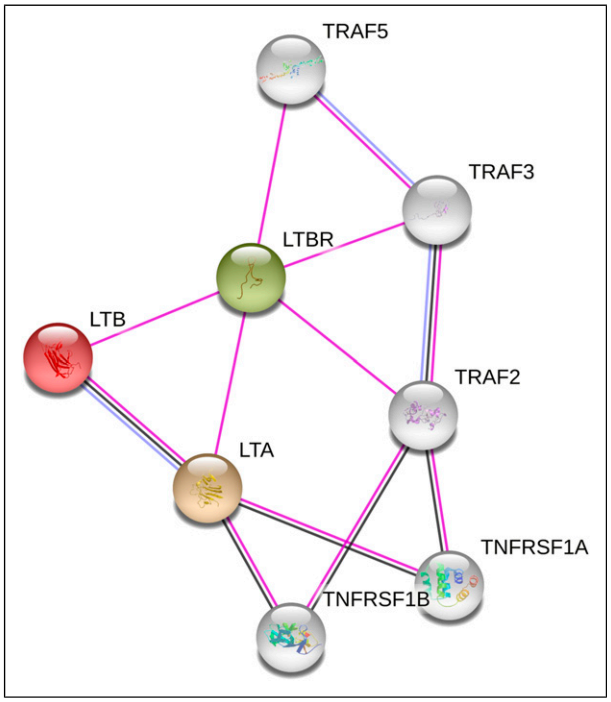

Figure 3. Protein-protein interaction network of predicted functional proteins for LTB. Network interaction representation for LTB was generated using the STRING database (Szklarczyk et al., 2019). Edges represent specific or meaningful associations. The colors of the edges connecting the nodes represent the types of evidence supporting the connections, namely: known interactions from experimental evidence (pink), predicted gene co-occurrence (blue), and co-expression (black).
Note. LTA = lymphotoxin alpha; LTB = lymphotoxin beta; LTBR = lymphotoxin beta receptor; TNFRSF1A = tumor necrosis factor receptor super family 1 A; TNFRSF1B = tumor necrosis factor receptor super family 1 B; TRAF2 = tumor necrosis factor receptor associated factor 2; TRAF3 = tumor necrosis factor receptor associated factor 3; TRAF5 = tumor necrosis factor receptor associated factor 5.

2021). While LTA_1B_2 is expressed on the surface of lymphoid cells (e.g., B cells, natural killer cells, T cells), LTBR is expressed on stromal and myeloid cells (e.g., dendritic cells, macrophages). Signaling between these distinct cell types is important for the formation and maintenance of lymphoid tissue. While notable for its role in embryonic lymph node and Peyer's patch formation; splenic structure maintenance; and lymph node homeostasis (Sedy et al., 2014), LTB may play a role in the regulation of mucosal immune responses of the gastrointestinal tract (Upadhyay & Fu, 2013).

From its position at the beginning of the NF-κB signaling pathway, LTB can induce NF-κB signaling and inflammatory response(s). As illustrated in Figure 3, LTB, LTA, and LTBR form a close, interacting network with three TNF receptor associated factors (TRAFs; i.e., TRAF2, TRAF3, TRAF5) and two major receptors for TNF (i.e., TNF receptor super family (TNFRSF) 1A, TNFRSF1B). These TRAFs are intracellular signaling molecules that regulate the canonical and non-canonical pathways that lead to NF-κB activation (Shi & Sun, 2018). In addition, TNFRSF1A and TNFRSF1B are receptors for LTA_3 and are involved in the canonical NF-κB signaling pathway and mediation of apoptosis (Ware, 2005).

Role of LTB in Intestinal Inflammation

While no pre-clinical or clinical study has evaluated for associations between LTB and gastrointestinal symptoms associated with chemotherapy administration, LTA, LTB, and LTBR appear to be involved in the mechanisms that underlie inflammatory bowel disease (Gubernatorova & Tumanov, 2016). For example, in a study that investigated the mechanisms by which activation of LTBR signaling influences acute inflammation in a mouse model of acute colitis induced by dextrose sulfate sodium (DSS; Jungbeck et al., 2008), comparisons of inflammatory responses in colonic tissue were performed using three models of LTBR signaling ablation (i.e., antibody binding to the receptor, LTBR-deficient mice, and LTAB-deficient mice). All three ablation models resulted in aggravation of the colitis and release of inflammatory cytokines. In addition, all of the mice lost weight. In a second study of chronic DSS-induced colitis (Stopfer et al., 2004), the expression of *Ltb* in colonic tissue was increased. Interestingly, inhibition of LTBR resulted in *decreases* in the development of inflammation as well as in the production of TNF, IL1B, and IL6 in colonic tissue. Taken together, these pre-clinical findings provide evidence of an association between LTB and inflammatory states in the bowel. In addition, these results suggest that the effects of LTB signaling may be different in the settings of acute versus chronic inflammation. Additional research is needed to determine the role of LTB signaling in the development and manifestations of chemotherapy-induced inflammatory responses in the gastrointestinal mucosa.

Two clinical studies have evaluated for differences in *LTB* expression in patients with and without inflammatory bowel disease (Agyekum et al., 2003; Pisani et al., 2022). In the first study (Agyekum et al., 2003), compared to healthy controls, the expression of *LTB* on lymphocytes and plasma cells was increased in the mucosa of the colonic tissue of patients with ulcerative colitis and in the ileum of patients with Crohn's disease. In the second study (Pisani et al., 2022), compared to healthy controls, *LTB*, C-C motif chemokine ligand (*CCL*)*19*, and *CCL21* were differentially expressed and upregulated in the colonic tissue of patients with microscopic colitis. These findings were confirmed in intestinal tissue from an independent sample of patients with microscopic colitis. These findings support the role of LTB signaling in gastrointestinal inflammation.

Nausea and a Gastrointestinal Cluster

While additional research is needed to evaluate the role of LTB in a gastrointestinal cluster in oncology patients receiving

chemotherapy, our findings build on previous studies of patients who overlapped with the samples used in this analysis (Papachristou et al., 2019; Singh et al., 2021; Singh, Kober, et al., 2020; Singh, Dhruva, et al., 2020). Two studies evaluated for differentially perturbed pathways between oncology patients with and without chemotherapy-induced nausea (Singh et al., 2021; Singh, Dhruva, et al., 2020). In addition to the NF-κB signaling pathway (Singh, Dhruva, et al., 2020), two additional pathways that are implicated in gastrointestinal inflammation (Cinausero et al., 2017) were differentially perturbed between patients with and without nausea (i.e., apoptosis (Singh et al., 2021), cytokine-cytokine signaling (Singh, Dhruva, et al., 2020)).

While these previous studies focused on a single symptom (Singh et al., 2021; Singh, Dhruva, et al., 2020), nausea is associated with the co-occurrence of several other gastrointestinal symptoms. For example, in another study by our team (Singh, Kober, et al., 2020), compared to patients without nausea, patients with nausea were more likely to report the occurrence of 11 additional gastrointestinal symptoms. Of note, nine of these symptoms (i.e., abdominal cramps, change in the way food tastes, constipation, diarrhea, difficulty swallowing, dry mouth, lack of appetite, weight loss, vomiting) were identified in our gastrointestinal cluster. While these findings are not surprising given that patients overlapped across the different analyses, similar findings were reported elsewhere. For example, in a study that evaluated the severity of 22 symptoms in patients with ovarian cancer receiving chemotherapy (Donovan et al., 2016), nausea was associated with five symptoms that were identified in our gastrointestinal cluster (i.e., bowel disturbances, dizziness, lack of appetite, vomiting, weight loss).

In another study by our team that identified a gastrointestinal cluster using network analysis (Papachristou et al., 2019), nausea was identified as the most important symptom within the network (i.e., theoretically has the greatest impact on other symptoms). We suggested that alleviating nausea may reduce the occurrence of the other symptoms within the network. Taken together, these findings suggest that nausea may be a sentinel symptom that drives the occurrence of other gastrointestinal symptoms.

Limitations and Future Directions

While these data provide preliminary evidence to support the hypothesis that the trans eCpG locus cg03171795 is involved in regulatory processes, it is not entirely clear how hypermethylation of this trans eCpG locus regulates the expression of *LTB*. An association between the methylation state of cg03171795 and expression of *LTB* was identified by Kennedy et al. (2018) in their genome-wide association study that tested for associations between CpG methylation and gene expression using data from the Multi-Ethnic Study of Atherosclerosis. Notably, trans eCpGs were identified more often than was expected and were more common than cis or distal eCpGs. The authors hypothesized that methylation regulates gene expression largely through secondary regulatory mechanisms, such as enhancer CpGs rather than promoter CpGs (Kennedy et al., 2018). Given that the regulatory role of trans eCpGs is an understudied area of epigenomic research, ongoing research is warranted. In vitro analyses may shed light on the indirect regulatory role(s) that hypermethylation of cg03171795 has on the expression of *LTB* (Rakyan et al., 2011). In addition, future research is warranted to evaluate the functional effect of *LTB* and other genes in the NF-κB signaling pathway in patients receiving chemotherapy. A multi-staged data-integrated multi-omics analysis (Harris et al., 2021) that evaluates for associations between a gastrointestinal cluster and differentially expressed genes (i.e., RNA, proteins) can shed light on the functional effect of these genes.

While an association between the trans eCpG locus cg03171795 and *LTB* was identified in the EPIC sample, this association was not found in our 450K sample. This lack of validation may be due to heterogeneity between the samples (e.g., gender, cancer type); the relatively small sample size (n = 146); and/or too small an effect size to identify a relationship. Additional research is needed to validate this association in patients receiving chemotherapy. In addition, given the study's cross-sectional design, evaluation of changes in levels of methylation for cg03171795 and *LTB* expression throughout chemotherapy is warranted. Given the statistical challenges with the distribution of the gastrointestinal cluster factor scores, the best methods to incorporate the use of symptom cluster factor scores in methylation analyses need to be determined.

Conclusion

This exploratory study is the first to evaluate for associations between a gastrointestinal symptom cluster and changes in epigenetic regulation of an inflammatory mechanism in oncology patients receiving chemotherapy. Our finding suggests that the occurrence of a gastrointestinal cluster is associated with increased expression of *LTB* through hypermethylation of one trans eCpG locus. This finding provides new evidence to support the hypothesis that NF-κB signaling results in a variety of gastrointestinal symptoms (Cinausero et al., 2017; Sonis, 2002; Sonis et al., 2004) and suggests directions for additional mechanistic studies.

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Author Contributions

Dr. Harris contributed to the study conception and design; contributed to the data analysis and interpretation; drafted and critically revised

the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Drs. Miaskowski and Kober contributed to the study conception and design; contributed to the data acquisition, analysis, and interpretation; drafted and critically revised the manuscript; gave final approval; and agree to be accountable for all aspects of work ensuring integrity and accuracy. Drs. Conley, Hammer, Dhruva, and Levine contributed to the data interpretation; critically revised the manuscript; gave final approval; and agree to be accountable for all aspects of work ensuring integrity and accuracy. Dr. Olshen contributed to analysis and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

References

Agyekum, S., Church, A., Sohail, M., Krausz, T., Van Noorden, S., Polak, J., & Cohen, J. (2003). Expression of lymphotoxin-beta (LT-beta) in chronic inflammatory conditions. *The Journal of Pathology*, *199*(1), 115–121. https://doi.org/10.1002/path.1249

Andersson, R., Sandelin, A., & Danko, C. G. (2015). A unified architecture of transcriptional regulatory elements. *Trends in Genetics*, *31*(8), 426–433. https://doi.org/10.1016/j.tig.2015.05.007

Aryee, M. J., Jaffe, A. E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A. P., Hansen, K. D., & Irizarry, R. A. (2014). Minfi: A flexible and comprehensive bioconductor package for the analysis of infinium DNA methylation microarrays. *Bioinformatics*, *30*(10), 1363–1369. https://doi.org/10.1093/bioinformatics/btu049

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bock, C. (2012). Analysing and interpreting DNA methylation data. *Nature Reviews Genetics*, *13*(10), 705–719. https://doi.org/10.1038/nrg3273

Borelli, A., & Irla, M. (2021). Lymphotoxin: From the physiology to the regeneration of the thymic function. *Cell Death & Differentiation*, *28*(8), 2305–2314. https://doi.org/10.1038/s41418-021-00834-8

Bowen, J. M., Gibson, R. J., Tsykin, A., Stringer, A. M., Logan, R. M., & Keefe, D. M. (2007). Gene expression analysis of multiple gastrointestinal regions reveals activation of common cell regulatory pathways following cytotoxic chemotherapy. *International Journal of Cancer*, *121*(8), 1847–1856. https://doi.org/10.1002/ijc.22895

Brown, T. (2015). *The common factor model and exploratory factor analysis* (2nd ed.). The Guilford Press.

Carlotto, A., Hogsett, V. L., Maiorini, E. M., Razulis, J. G., & Sonis, S. T. (2013). The economic burden of toxicities associated with cancer treatment: Review of the literature and analysis of nausea and vomiting, diarrhoea, oral mucositis and fatigue. *PharmacoEconomics*, *31*(9), 753–766. https://doi.org/10.1007/s40273-013-0081-2

Chen, M. L., & Lin, C. C. (2007). Cancer symptom clusters: A validation study. *Journal of Pain & Symptom Management*, *34*(6), 590–599. https://doi.org/10.1016/j.jpainsymman.2007.01.008

Chen, Y. A., Lemire, M., Choufani, S., Butcher, D. T., Grafodatskaya, D., Zanke, B. W., Gallinger, S., Hudson, T. J., & Weksberg, R. (2013). Discovery of cross-reactive probes and polymorphic CpGs in the illumina infinium humanmethylation450 microarray. *Epigenetics*, *8*(2), 203–209. https://doi.org/10.4161/epi.23470

Cinausero, M., Aprile, G., Ermacora, P., Basile, D., Vitale, M. G., Fanotto, V., Parisi, G., Calvetti, L., & Sonis, S. T. (2017). New frontiers in the pathobiology and treatment of cancer regimen-related mucosal injury. *Frontiers in Pharmacology*, *8*, 354. https://doi.org/10.3389/fphar.2017.00354

Donovan, H. S., Hagan, T. L., Campbell, G. B., Boisen, M. M., Rosenblum, L. M., Edwards, R. P., Bovbjerg, D. H., & Horn, C. C. (2016). Nausea as a sentinel symptom for cytotoxic chemotherapy effects on the gut-brain axis among women receiving treatment for recurrent ovarian cancer: An exploratory analysis. *Supportive Care in Cancer*, *24*(6), 2635–2642. https://doi.org/10.1007/s00520-015-3071-4

Du, P., Kibbe, W. A., & Lin, S. M. (2008). Lumi: A pipeline for processing illumina microarray. *Bioinformatics*, *24*(13), 1547–1548. https://doi.org/10.1093/bioinformatics/btn224

Du, P., Zhang, X., Huang, C., Jafari, N., Kibbe, W. A., Hou, L., & Lin, S. M. (2010). Comparison of beta-value and M-value

methods for quantifying methylation levels by microarray analysis. *Bioinformatics*, *1*(587), 1–9. https://doi.org/10.1186/1471-2105-11-587

ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature*, *489*(7414), 57–74. https://doi.org/10.1038/nature11247

Ernst, J., Kheradpour, P., Mikkelsen, T. S., Shoresh, N., Ward, L. D., Epstein, C. B., Zhang, X., Wang, L., Issner, R., Coyne, M., Ku, M., Durham, T., Kellis, M., & Bernstein, B. E. (2011). Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature*, *473*(7345), 43–49. https://doi.org/10.1038/nature09906

Extermann, M., Bonetti, M., Sledge, G. W., O'Dwyer, P. J., Bonomi, P., & Benson, A. B., 3rd (2004). MAX2 – A convenient index to estimate the average per patient risk for chemotherapy toxicity; validation in ECOG trials. *European Journal of Cancer*, *40*(8), 1193–1198. https://doi.org/10.1016/j.ejca.2004.01.028

Gibson, R. J., & Keefe, D. M. (2006). Cancer chemotherapy-induced diarrhoea and constipation: Mechanisms of damage and prevention strategies. *Supportive Care in Cancer*, *14*(9), 890–900. https://doi.org/10.1007/s00520-006-0040-y

Gubernatorova, E. O., & Tumanov, A. V. (2016). Tumor necrosis factor and lymphotoxin in regulation of intestinal inflammation. *Biochemistry (Mosc)*, *81*(11), 1309–1325. https://doi.org/10.1134/S0006297916110092

Harris, C. S., Miaskowski, C. A., Dhruva, A. A., Cataldo, J., & Kober, K. M. (2021). Multi-staged data-integrated multi-omics analysis for symptom science research. *Biological Research for Nursing*, *23*(4), 596–607. https://doi.org/10.1177/10998004211003980

Harris, C. S., Kober, K. M., Conley, Y. P., Dhruva, A. A., Hammer, M., & Miaskowski, C. A. (2022). Symptom clusters in patients receiving chemotherapy: A systematic review. *BMJ Supportive & Palliative Care*, *12*(1), 10–21. https://doi.org/10.1136/bmjspcare-2021-003325

Harris, C. S., Kober, K. M., Cooper, B., Conley, Y. P., Dhruva, A. A., Hammer, M. J., Paul, S., Levine, J. D., & Miaskowski, C. A. (2022). Symptom clusters in outpatients with cancer using different dimensions of the symptom experience. *Supportive Care in Cancer*, *30*(8), 6889–6899. https://doi.org/10.1007/s00520-022-07125-z

Jones, M. J., Goodman, S. J., & Kobor, M. S. (2015). DNA methylation and healthy human aging. *Aging Cell*, *14*(6), 924–932. https://doi.org/10.1111/acel.12349

Jones, P. A. (2012). Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nature Reviews Genetics*, *13*(7), 484–492. https://doi.org/10.1038/nrg3230

Jungbeck, M., Stopfer, P., Bataille, F., Nedospasov, S. A., Mannel, D. N., & Hehlgans, T. (2008). Blocking lymphotoxin beta receptor signalling exacerbates acute DSS-induced intestinal inflammation—opposite functions for surface lymphotoxin expressed by T and B lymphocytes. *Molecular Immunology*, *45*(1), 34–41. https://doi.org/10.1016/j.molimm.2007.05.007

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, *28*(1), 27–30. https://doi.org/10.1093/nar/28.1.27

Karnofsky, D. (1977). Performance scale. In G. Kennealey, & M. Mitchell (Eds.), *Factors that influence the therapeutic response in cancer: A comprehensive treatise*. Plenum Press.

Karnuta, J. M., & Scacheri, P. C. (2018). Enhancers: Bridging the gap between gene control and human disease. *Human Molecular Genetics*, *27*(R2), R219–R227. https://doi.org/10.1093/hmg/ddy167

Kennedy, E. M., Goehring, G. N., Nichols, M. H., Robins, C., Mehta, D., Klengel, T., Eskin, E., Smith, A. K., & Conneely, K. N. (2018). An integrated -omics analysis of the epigenetic landscape of gene expression in human blood cells. *BMC Genomics*, *19*(1), 476. https://doi.org/10.1186/s12864-018-4842-3

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., & Haussler, D. (2002). The human genome browser at UCSC. *Genome Research*, *12*(6), 996–1006. https://doi.org/10.1101/gr.229102

Kober, K., Lee, M.-C., Olshen, A., Conley, Y., Sirota, M., Keiser, M., Hammer, M., Abrams, G., Schumacher, M., Levine, J., & Miaskowski, C. (2020). Differential methylation and expression of genes in the hypoxia inducible factor 1 (HIF-1) signaling pathway are associated with paclitaxel-induced peripheral neuropathy in breast cancer survivors and with preclinical models of chemotherapy-induced neuropathic pain. *Molecular Pain*, *16*(1744806920936502), 174480692093650. https://doi.org/10.1177/1744806920936502

Leek, J. T., & Storey, J. D. (2007). Capturing heterogeneity in gene expression studies by surrogate variable analysis. *Plos Genetics*, *3*(9), 1724–1735. https://doi.org/10.1371/journal.pgen.0030161

Matzka, M., Köck-Hódi, S., Jahn, P., & Mayer, H. (2018). Relationship among symptom clusters, quality of life, and treatment-specific optimism in patients with cancer. *Supportive Care in Cancer*, *26*(8), 2685–2693. https://doi.org/10.1007/s00520-018-4102-8

McGregor, K., Bernatsky, S., Colmegna, I., Hudson, M., Pastinen, T., Labbe, A., & Greenwood, C. M. (2016). An evaluation of methods correcting for cell-type heterogeneity in DNA methylation studies. *Genome Biology*, *17*(1), 84. https://doi.org/10.1186/s13059-016-0935-y

Molassiotis, A., Wengstrom, Y., & Kearney, N. (2010). Symptom cluster patterns during the first year after diagnosis with cancer. *Journal of Pain & Symptom Management*, *39*(5), 847–858. https://doi.org/10.1016/j.jpainsymman.2009.09.012

Muthén, L., & Muthén, B. (2019). *Mplus* (Version 8.4). Muthen & Muthen.

Oeckinghaus, A., & Ghosh, S. (2009). The NF-kappaB family of transcription factors and its regulation. *Cold Spring Harbor Perspectives in Biology*, *1*(4), a000034. https://doi.org/10.1101/cshperspect.a000034

Papachristou, N., Barnaghi, P., Cooper, B., Kober, K. M., Maguire, R., Paul, S. M., Hammer, M., Wright, F., Armes, J., Furlong, E. P., McCann, L., Conley, Y. P., Patiraki, E., Katsaragakis, S., Levine, J. D., & Miaskowski, C. (2019). Network analysis of the multidimensional symptom experience of oncology. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-018-36973-1

Pirri, C., Bayliss, E., Trotter, J., Olver, I. N., Katris, P., Drummond, P., & Bennett, R. (2013). Nausea still the poor relation in antiemetic therapy? The impact on cancer patients' quality of life and psychological adjustment of nausea, vomiting and appetite loss, individually and concurrently as part of a symptom cluster. *Supportive Care in Cancer*, *21*(3), 735–748. https://doi.org/10.1007/s00520-012-1574-9

Pisani, L. F., Tontini, G., Vecchi, M., Croci, G. A., & Pastorelli, L. (2022). NF-kB pathway is involved in microscopic colitis pathogenesis. *The Journal of International Medical Research*, *50*(3), 3000605221080104. https://doi.org/10.1177/03000605221080104

Portela, A., & Esteller, M. (2010). Epigenetic modifications and human disease. *Nature Biotechnology*, *28*(10), 1057–1068. https://doi.org/10.1038/nbt.1685

Portenoy, R. K., Thaler, H. T., Kornblith, A. B., Lepore, J. M., Friedlander-Klar, H., Kiyasu, E., Sobel, K., Coyle, N., Kemeny, N., Norton, L., & Scher, H. (1994). The memorial symptom assessment scale: An instrument for the evaluation of symptom prevalence, characteristics and distress. *European Journal of Cancer*, *30A*(9), 1326–1336. https://doi.org/10.1016/0959-8049(94)90182-1

Rakyan, V. K., Down, T. A., Balding, D. J., & Beck, S. (2011). Epigenome-wide association studies for common human diseases. *Nature Reviews Genetics*, *12*(8), 529–541. https://doi.org/10.1038/nrg3000

Ren, H., Tang, P., Zhao, Q., & Ren, G. (2017). Symptom clusters and related factors in bladder cancer patients three months after radical cystectomy. *BMC Urology*, *17*(1), 65. https://doi.org/10.1186/s12894-017-0255-x

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7), e47. https://doi.org/10.1093/nar/gkv007

Rosenbloom, K. R., Sloan, C. A., Malladi, V. S., Dreszer, T. R., Learned, K., Kirkup, V. M., Wong, M. C., Maddren, M., Fang, R., Heitner, S. G., Lee, B. T., Barber, G. P., Harte, R. A., Diekhans, M., Long, J. C., Wilder, S. P., Zweig, A. S., Karolchik, D., Kuhn, R. M., Haussler, D., & Kent, W. J. (2013). ENCODE data in the UCSC genome browser: Year 5 update. *Nucleic Acids Research*, *41*(D1), D56–D63. https://doi.org/10.1093/nar/gks1172

Salas, L., & Koestler, D. (2021). FlowSorted.Blood.EPIC: Illumina EPIC data on immunomagnetic sorted peripheral adult blood cells. (Version 1.12.1) R package. https://github.com/immunomethylomics/FlowSorted.Blood.EPIC

Salas, L. A., Koestler, D. C., Butler, R. A., Hansen, H. M., Wiencke, J. K., Kelsey, K. T., & Christensen, B. C. (2018). An optimized library for reference-based deconvolution of whole-blood biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. *Genome Biology*, *19*(1), 64. https://doi.org/10.1186/s13059-018-1448-7

Sangha, O., Stucki, G., Liang, M. H., Fossel, A. H., & Katz, J. N. (2003). The self-administered comorbidity questionnaire: A new method to assess comorbidity for clinical and health services research. *Arthritis & Rheumatism*, *49*(2), 156–163. https://doi.org/10.1002/art.10993

Sedy, J., Bekiaris, V., & Ware, C. F. (2014). Tumor necrosis factor superfamily in innate immunity and inflammation. *Cold Spring Harbor Perspectives in Biology*, *7*(4), a016279. https://doi.org/10.1101/cshperspect.a016279

Shi, J. H., & Sun, S. C. (2018). Tumor necrosis factor receptor-associated factor regulation of nuclear factor kappaB and mitogen-activated protein kinase pathways. *Frontiers in Immunology*, *9*(■), 1849. https://doi.org/10.3389/fimmu.2018.01849

Singh, K. P., Dhruva, A., Flowers, E., Paul, S. M., Hammer, M. J., Wright, F., Cartwright, F., Conley, Y. P., Melisko, M., Levine, J. D., Miaskowski, C., & Kober, K. M. (2020). Alterations in patterns of gene expression and perturbed pathways in the gut-brain axis are associated with chemotherapy-induced nausea. *Journal of Pain & Symptom Management*, *59*(6), 1248–1259. https://doi.org/10.1016/j.jpainsymman.2019.12.352

Singh, K., Kober, K. M., Paul, S. M., Hammer, M., Wright, F., Conley, Y. P., Levine, J. D., & Miaskowski, C. (2020). Gastrointestinal symptoms are associated with trajectories of chemotherapy-induced nausea. *Supportive Care in Cancer*, *28*(5), 2205–2215. https://doi.org/10.1007/s00520-019-05031-5

Singh, K., Cao, H., Miaskowski, C., Conley, Y. P., Hammer, M., Wright, F., Levine, J. D., & Kober, K. M. (2021). Perturbations in endocytotic and apoptotic pathways are associated with chemotherapy-induced nausea. *Biological Research for Nursing*, *23*(2), 238–247. https://doi.org/10.1177/1099800420951271

Skerman, H. M., Yates, P. M., & Battistutta, D. (2012). Cancer-related symptom clusters for symptom management in outpatients after commencing adjuvant chemotherapy, at 6 months, and 12 months. *Supportive Care in Cancer*, *20*(1), 95–105. https://doi.org/10.1007/s00520-010-1070-z

Sonis, S. T., Elting, L. S., Keefe, D., Peterson, D. E., Schubert, M., Hauer-Jensen, M., Bekele, B. N., Raber-Durlacher, J., Donnelly, J. P., & Rubenstein, E. B. &, Mucositis Study Section of the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology (2004). Perspectives on cancer therapy-induced mucosal injury: Pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer*, *100*(9 Suppl), 1995–2025. https://doi.org/10.1002/cncr.20162

Sonis, S., Haddad, R., Posner, M., Watkins, B., Fey, E., Morgan, T. V., Mookanamparambil, L., & Ramoni, M. (2007). Gene expression changes in peripheral blood cells provide insight into the biological mechanisms associated with regimen-related toxicities in patients being treated for head and neck cancers. *Oral Oncology*, *43*(3), 289–300. https://doi.org/10.1016/j.oraloncology.2006.03.014

Sonis, S. T. (2002). Biologic role for nuclear factor-kappa B in disease and its potential involvement in mucosal injury associated with anti-neoplastic therapy. *Critical Reviews in Oral Biology and Medicine*, *13*(5), 380–389. https://doi.org/10.1177/154411130201300502

Stopfer, P., Obermeier, F., Dunger, N., Falk, W., Farkas, S., Janotta, M., Moller, A., Mannel, D. N., & Hehlgans, T. (2004). Blocking

lymphotoxin-beta receptor activation diminishes inflammation via reduced mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expression and leucocyte margination in chronic DSS-induced colitis. *Clinical & Experimental Immunology*, *136*(1), 21–29. https://doi.org/10.1111/j.1365-2249.2004.02402.x

Sullivan, C. W., Leutwyler, H., Dunn, L. B., & Miaskowski, C. (2018). A review of the literature on symptom clusters in studies that included oncology patients receiving primary or adjuvant chemotherapy. *Journal of Clinical Nursing*, *27*(3–4), 516–545. https://doi.org/10.1111/jocn.14057

Suwisith, N., Hanucharurnkul, S., Dodd, M., Vorapongsathorn, T., Pongthavorakamol, K., & Asavametha, N. (2008). Symptom clusters and functional status of women with breast cancer. *Thai Journal of Nursing Research*, *12*(3), 153–165. https://doi.org/10.1016/j.ejon.2009.09.005

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Mering, C. V. (2019). STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, *47*(D1), D607–D613. https://doi.org/10.1093/nar/gky1131

Upadhyay, V., & Fu, Y. X. (2013). Lymphotoxin signalling in immune homeostasis and the control of microorganisms. *Nature Reviews. Immunology*, *13*(4), 270–279. https://doi.org/10.1038/nri3406

Ware, C. F. (2005). Network communications: Lymphotoxins, LIGHT, and TNF. *Annual Review of Immunology*, *23*(1), 787–819. https://doi.org/10.1146/annurev.immunol.23.021704.115719

Yang, G. S., Mi, X., Jackson-Cook, C. K., Starkweather, A. R., Lynch Kelly, D., Archer, K. J., Zou, F., & Lyon, D. E. (2020). Differential DNA methylation following chemotherapy for breast cancer is associated with lack of memory improvement at one year. *Epigenetics*, *15*(5), 499–510. https://doi.org/10.1080/15592294.2019.1699695