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Epigenetic Regulation of Inflammatory Mechanisms and a Psychological Symptom Cluster in Patients Receiving Chemotherapy

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Abstract

Background: A psychological symptom cluster is the most common cluster identified in oncology patients. While inflammatory mechanisms are hypothesized to underlie this cluster, epigenetic contributions are unknown.

Objectives: This study's purpose was to evaluate associations between the occurrence of a psychological symptom cluster and levels of DNA methylation for inflammatory genes in a heterogeneous sample of patients with cancer receiving chemotherapy.

Methods: Prior to their second or third cycle of chemotherapy, 1,071 patients reported the occurrence of 38 symptoms using the Memorial Symptom Assessment Scale. A psychological cluster was identified using exploratory factor analysis. Differential methylation analyses were performed in two independent samples using Illumina Infinium 450K and EPIC microarrays. Expression-associated CpG (eCpG) loci in the promoter region of 114 inflammatory genes on the 450K and 112 genes on the EPIC microarray were evaluated for associations with the psychological cluster. Robust Rank Aggregation was used to identify differentially methylated genes across both samples. Significance was assessed using a false discovery rate of .05 under the Benjamini-Hochberg procedure.

Results: Cluster of differentiation 40 (*CD40*) was differentially methylated across both samples. All six promoter eCpGs for *CD40* that were identified across both samples were hypomethylated in the psychological cluster group.

Conclusions: This study is the first to suggest associations between a psychological symptom cluster and differential DNA methylation of a gene involved in tissue inflammation and cell-mediated immunity. Our findings suggest that increased *CD40* expression through hypomethylation of promoter eCpG loci is involved in the occurrence of a psychological symptom cluster in patients receiving chemotherapy. These findings suggest a direction for mechanistic studies.

Keywords

cancer; chemotherapy; DNA methylation; inflammation; psychological symptom cluster

A psychological symptom cluster is the most common cluster identified in patients receiving chemotherapy (Harris, Kober, Conley et al., 2022). This cluster is observed across cancer types, persists over time, and is stable across various dimensions of the symptom experience (Harris, Kober, Conley et al., 2022; Harris, Kober, Cooper et al., 2022). In addition, the psychological symptom cluster (hereafter referred to as psychological cluster) is associated with decrements in functional status and quality of life (Chen et al., 2021). This cluster consisted of primarily anxious (i.e., worrying, feeling nervous, feeling irritable) and depressive (i.e., feeling sad, difficulty concentrating, "I don't look like myself"; American Psychiatric Association, 2013) symptoms (Figure 1; Harris, Kober, Cooper et al., 2022). Individually, clinical or subclinical levels of anxiety and depression occur in 41.6% and 29.4% of oncology patients, respectively (Linden et al., 2012). However, 34.0% of oncology patients experience both symptoms (Brown et al., 2010). This finding suggests a strong

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association between these two symptoms and explains their inclusion in a psychological cluster.

Given the strong relationship between these two symptoms and the ubiquitous nature and negative effect of the psychological cluster on patients with cancer, investigation into the mechanism(s) that underlie this cluster is warranted. Both anxiety (Costello et al., 2019) and depressive disorders (Dantzer et al., 2008; Osimo et al., 2020) are associated with inflammatory processes. However, studies on the associations between concurrent anxiety and depressive symptoms and inflammatory markers in oncology patients are limited. In a series of two studies that used the Hospital Anxiety and Depression Scale (HADS) to identify colorectal cancer patients with concurrent anxiety and depression (i.e., HADS total score of > 19; Miranda et al., 2018; Oliveira Miranda et al., 2014), associations between group membership and serum levels of a number of cytokines (i.e., interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor [TNF]-α, transforming growth factor [TFG]-β) were evaluated. Across both studies, in the patients with concurrent anxiety and depression, higher HADS scores were associated with increased levels of IL-1β, IL-6, IL-8, and TNF-α and lower serum levels of IL-10. No associations were found between IL-12 and TGF-β. While these findings support an association between the co-occurrence of anxiety and depression and inflammatory mechanisms in oncology patients, the sample sizes were very small—only patients with colorectal cancer were included—and only seven cytokines were evaluated. Given these limitations, additional research is warranted on the relationships between psychological symptoms and inflammatory mechanisms.

DNA methylation is an epigenetic mechanism that regulates gene expression by adding or removing methyl groups at the 5’-position of cytosine residues (Gibney & Nolan, 2010). DNA methylation can be used to evaluate changes in gene regulation that occur in response to environmental stimuli and stressors (Stephens et al., 2013). While the physiological and psychological stress associated with a cancer diagnosis and its treatments can affect the epigenome (Lyon et al., 2014), less is known about its effect on symptom burden. Previous research in oncology patients found that fatigue was associated with epigenetic changes in genes involved in inflammatory processes or immune function (Flowers et al., 2019). An increased understanding of the associations between a psychological cluster and epigenetic regulation of inflammatory processes may provide insights into its underlying mechanism(s). In addition, DNA methylation is potentially modifiable, making it a potential target for therapeutic interventions (Szyf, 2005).

In patients without cancer, recent evidence suggests that anxiety (Emeny et al., 2018) and depressive disorders (Kim et al., 2016) are associated with methylation of inflammatory genes. For example, in a population-based cohort study that compared individuals with no or minimal anxiety to those with severe anxiety (Emeny et al., 2018), increased methylation of a single CpG locus in the promoter region of the ankyrin repeat and suppressor of cytokine signaling box containing 1 (*ASB1*) gene was associated with being in the severe anxiety group. This finding was confirmed in an independent sample of patients with anxiety disorders. Of note, the product of this gene is involved in regulating cytokine signaling.

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In terms of depressive symptoms, a cohort study of elderly men evaluated for associations between depression scores and levels of methylation in CpG-rich promoter regions of seven genes involved in immune or inflammatory processes (Kim et al., 2016). Higher depression scores were associated with higher average promoter methylation of coagulation factor III (*F3*) and intercellular adhesion molecule-1 (*ICAM-1*). However, no associations were found with serum levels of ICAM-1.

While in a previous review (Lyon et al., 2014), epigenetic modifications were hypothesized to play a role in the development of psychological symptoms in oncology patients, no study has evaluated associations between a psychological cluster and epigenetic regulation of inflammatory mechanisms. Therefore, in a sample of outpatients receiving chemotherapy this study aimed to evaluate associations between a psychological cluster and DNA methylation levels using a panel of inflammatory genes.

Methods

Patients and Settings

This analysis is part of a larger study evaluating 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 boards at each of the study sites. Of the 2,234 patients approached, 1,343 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 research team member 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 used in this analysis. At enrollment, a total of 1,071 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:

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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 created the symptom clusters. The validity and reliability of the MSAS are well-established (Portenoy et al., 1994).

Phenotypic Analyses

Descriptive statistics and frequency distributions were calculated for the demographic and clinical characteristics using the IBM SPSS Statistics (Version 27). 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 of 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.

These methods identified a psychological cluster (Figure 1; Harris, Kober, Cooper et al., 2022). A factor score was calculated as the sum of the occurrence rates for the six symptoms in this cluster (range of 0 to 6). Initially, the DNA methylation analyses were conducted using the patients' symptom cluster factor scores as continuous values. 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 psychological cluster group vs. 1 to 6 symptoms = psychological cluster group).

Selection of DNA Methylation Loci

To evaluate the hypothesis that inflammatory mechanisms may underlie a psychological cluster, a comprehensive list of 1,027 genes involved in immune and inflammatory processes (e.g., cytokine signaling, nuclear factor kappa B [NF-κB] signaling) was identified from the literature (Loza et al., 2007). Then, CpG sites that reside in the promoter region of these genes and are known to have methylation values associated with changes in gene expression (Kennedy et al., 2018; i.e., expression-associated CpG [eCpG]) were used in our analyses.

Biospecimen Processing, Quantification of Methylation Status, and Quality Control

Methods for the 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 nanograms per microliter. DNA was bisulfite converted using the Zymo EZ-96 DNA

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Methylation Kit (Catalog #D5004) Deep-Well Format (Zymo Research, Irvine, CA) and used as input for the Illumina Infinium HD Methylation Assay (Illumina, San Diego, CA).

Funding for the methylation analyses was received at two different points in time; initially, to conduct a pilot study in women with breast cancer and later for the remaining patients. Therefore, of the 1,071 patients in this study, DNA methylation was measured for 146 patients using the Infinium HumanMethylation 450 BeadChip (i.e., 450K microarray sample) and for 925 patients using the Infinium MethylationEPIC BeadChip (i.e., EPIC microarray sample; Illumina, Inc., San Diego, CA). All 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 with < 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 1.40.0; Aryee et al., 2014; Du et al., 2008). Probes containing a single nucleotide polymorphism at a CpG or flanking site and probes 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 1.12.1; Salas & Koestler, 2018). Cell type deconvolution was performed using the IDOL L-DMR library for a cluster of differentiation 8 (CD8) and CD4 T-cells, natural killer (NK) cells, B cells, monocytes, and neutrophils (Salas et al., 2018). Differences in estimates of cell type composition between the psychological 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 psychological 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 models. 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 nontechnical variations that contributed to heterogeneity in the sample that was not due to the psychological cluster group, age, or cell type.

To evaluate for associations between the psychological cluster group and methylation status of regulatory regions of inflammatory 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). For genes with multiple eCpG loci, to examine them as a region (Robinson et al., 2014), Fisher’s Combined Probability test was used to combine the DMP tests using their uncorrected *p*-values (Supplemental Figure 1; Mosteller & Fisher, 1948). Using this approach, all tests for differential methylation of

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loci within the promoter region of a given gene were represented by a single, uncorrected *p*-value.

In order to identify findings across the 450K and EPIC microarrays, we used RobustRankAggreg (Version 1.1; Kolde et al., 2012). Rank aggregation meta-analytic approaches are used with information retrieval, marketing, and high-throughput data sets to integrate data from multiple ranked lists (Lin, 2010). In addition, rank aggregation techniques are invariant to transformation and normalization and robust to outliers (Li et al., 2019). First, the gene lists from each sample were individually ranked using the uncorrected *p*-values from the differential methylation analyses. Then, the genes from both samples were integrated and evaluated based on their individual rankings on the combined gene list. Finally, each gene was assigned a single *p*-value based on how "better it was positioned in the ranked lists than was expected by chance" (Kolde et al., 2012, p.574). The significance of this ranked set of genes was assessed using a false discovery rate (FDR) of 0.05 under the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995).

To characterize the potential functional roles of these eCpGs, we identified the direction of expression associated with methylation levels as quantified by Kennedy et al. (2018) in their eCpG data set. In addition, we evaluated for evidence of regulatory elements in the region surrounding the 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 of genes with differentially methylated promoter 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 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 years (*SD* = 11.7; Table 1). Most patients were well-educated (*M* = 16.3, SD = 2.9 years), exercised regularly (75.7%), and had never smoked (72.4%). Patients had an average of 2.4 (*SD* = 1.4) comorbid conditions and a KPS score of 79.1 (*SD* = 11.6). The most common type of cancer was breast (99.3%), followed by gastrointestinal (0.7%). The majority of patients (76.7%) had received either chemotherapy, surgery, and/or radiation therapy. Patients reported 16.0 (*SD* = 7.8) concurrent symptoms before their second or third cycle of chemotherapy.

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 years (*SD* = 12.2; Table 2). Most patients were well-educated (*M* = 16.1, *SD* = 3.0 years), exercised on a regular basis (71.6%), and had never smoked (66.4%). Patients had an average of 2.4 (*SD* = 1.4) comorbid conditions and a KPS score of 80.4 (*SD* = 12.6). The most common type of cancer was breast (39.5%), followed by gastrointestinal (34.0%), gynecological (15.9%), and lung (10.5%). The majority of patients (73.3%) had received

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either chemotherapy, surgery, and/or radiation therapy. Patients reported 13.5 (*SD* = 7.1) concurrent symptoms before their second or third cycle of chemotherapy.

DNA Methylation Analyses

For the 450K microarray sample, the NK cell type composition estimate was associated with the psychological cluster group; it was included with age and one surrogate variable as covariates in the final model. For this sample, of the 1,027 inflammation-related genes that were identified as candidates (Supplemental Data File 1 available at https://zenodo.org/record/6618110; Loza et al., 2007), 283 eCpG loci across 114 genes were evaluated for differential methylation. Of note, three genes were unique to the 450K microarrays (i.e., Fc gamma receptor IIa, Janus kinase 2, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein eta).

No cell-type compositions were associated with the psychological cluster group for the EPIC microarray sample. Therefore, the final model included age and the two surrogate variables as covariates in. For this sample, 267 eCpG loci across 112 genes were tested for differential methylation. Of note, one gene was unique to the EPIC microarray (i.e., major histocompatibility complex, class I, A).

The robust rank aggregation method identified one differentially methylated gene across the 450K and EPIC microarray samples (i.e., *CD40*, FDR = 0.017; Table 3). All six eCpGs for *CD40* (i.e., cg22232207, cg06571407, cg17929951, cg21601405, cg01943874, cg11841529) that were identified across both the 450K and EPIC microarray samples were hypomethylated in the psychological cluster group (view Supplemental Data Files at https://zenodo.org/record/6618551).

Discussion

This exploratory study is the first to evaluate for changes in epigenetic regulation of inflammatory mechanisms underlying a psychological cluster in patients receiving chemotherapy. Our findings suggest that membership in the psychological cluster group is linked to increased expression of the *CD40* gene through hypomethylation of multiple promoter loci. These findings build on previous research that suggests that dysregulation of a variety of inflammatory processes contributes to the development of psychological symptoms (Costello et al., 2019; Dantzer et al., 2008; Emeny et al., 2018; Kim et al., 2016; Miranda et al., 2018; Oliveira Miranda et al., 2014; Osimo et al., 2020).

Regulatory Role of eCpGs in *CD40* Expression

One of the major challenges for methylation association studies is establishing a functional role for the identified epigenetic variation (Rakyan et al., 2011). Without evidence of a functional role, it is difficult to distinguish between epigenetic variation as a cause or consequence of the phenotype (i.e., symptom cluster). In this study, multiple lines of evidence support a regulatory role for the eCpG loci associated with the psychological cluster group. First, our results suggest that these six loci function together as a region. As illustrated in Figure 2, all six eCpG loci are in the promoter region of *CD40* and are within 250 base pairs of each other. This finding is notable because previous findings suggest

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that multiple CpG sites showing similar methylation patterns in a small region have shared regulatory functions (Rakyan et al., 2011). Second, given that all six eCpG loci share the same direction of expression (i.e., hypomethylation), it suggests that they act together. Third, in another study (Kennedy et al., 2018), methylation levels for all six loci were positively associated with increased expression of *CD40*, which suggests a direct functional role for these loci.

Furthermore, all six loci are located within putative regulatory regions, as evidenced by independent ENCODE experiments that identified multiple types of regulatory elements. These elements include histone protein marks that are associated with promoters or enhancers (ENCODE Project Consortium, 2012); DNase I hypersensitivity clusters that are characteristic of *cis*-regulatory elements (ENCODE Project Consortium, 2012) that make DNA more accessible to transcription (ENCODE Project Consortium, 2011); and clusters of transcription factor binding (ENCODE Project Consortium, 2011). Taken together, these lines of evidence provide strong support for the hypothesis that these six loci that are associated with psychological cluster group membership act together in the regulation of *CD40* expression.

Role of CD40 in Inflammatory Processes

CD40 is a costimulatory protein receptor and a member of the tumor necrosis factor receptor (TNFR) superfamily (Elgueta et al., 2009). Expression of *CD40* is stimulated by a variety of cytokines, including IL-3 and interferon (IFN)-γ (Tang et al., 2021). CD40 signaling plays a central role in tissue inflammation, humoral and cell-mediated immunity (Tang et al., 2021), and various autoimmune (e.g., irritable bowel disease, multiple sclerosis) and malignant conditions (Elgueta et al., 2009). Together with its ligand CD40LG, membrane-bound CD40 forms a stimulatory immune checkpoint involved in T cell-dependent B cell differentiation and activation (Tang et al., 2021). Specifically, the interaction between CD40 and CD40LG is needed for fundamental B cell functions, including cellular proliferation, apoptosis, immunoglobulin production, and isotype switching (Tang et al., 2021).

Situated at the beginning of the NF-κB signaling pathway, CD40 signaling induces the production of NF-κB, a family of transcription factors involved in inflammatory responses as well as cell proliferation and survival (Liu et al., 2017). Various TNFR-associated factors (TRAFs) bind to the cytoplasmic domain of CD40 intracellularly and mediate it signaling to activate the canonical and noncanonical pathways within the NF-κB signaling pathway (Tang et al., 2021). As illustrated in Supplemental Figure 2, the protein product of *CD40* interacts directly with five TRAFs (i.e., TRAF1, 2, 3, 5, 6) and baculoviral inhibitor of apoptosis repeat containing 2 (BIRC2), a regulator of apoptosis and inflammatory signaling (Zhou et al., 2013). These TRAFs activate or inhibit various signaling pathways (e.g., NF-κB, mitogen-activated protein kinase) and trigger the production of various inflammatory cytokines (e.g., IL-6, TNF-α; Elgueta et al., 2009).

Role of CD40 in Psychological Disorders and/or Symptoms

No studies have examined the relationships between anxiety and/or depressive symptoms and epigenetic regulation of *CD40* in oncology patients. However, multiple clinical

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(Neubauer et al., 2013; Nowak et al., 2019; Zahn et al., 2015) and preclinical (Cathomas et al., 2015; Müller et al., 2015) studies provide evidence to support associations between depression and changes in *CD40* expression and inflammatory responses. In two studies that evaluated associations between major depressive disorder (MDD) and inflammatory markers, platelet expression of CD40 was higher in patients newly diagnosed with MDD compared to healthy controls (Neubauer et al., 2013; Zahn et al., 2015). In another study that evaluated differences in plasma levels of pro-inflammatory cytokines and circulating monocytes in patients with MDD and suicidal ideation compared to healthy controls (Nowak et al., 2019), patients with MDD had significantly higher levels of activated CD40-expressing monocytes. In addition, these patients had increased plasma levels of IL-6 and IL-12.

Findings from two preclinical studies provide additional evidence to suggest that increased CD40 signaling is involved in developing depressive symptoms and inflammation (Cathomas et al., 2015; Müller et al., 2015). In these studies (Cathomas et al., 2015; Müller et al., 2015), the presence of depressive symptomatology was identified in mice by evaluating for specific behaviors (i.e., reduced saccharin preference or consumption indicated decreased interest or pleasure in activities; decreased weight indicated decreased appetite; decreased classical conditioning indicated cognitive impairment; decreased locomotor activity indicated sleep impairment). In the first study (Cathomas et al., 2015), compared to untreated controls, mice treated with a CD40 agonist antibody exhibited symptoms characteristic of depressive symptomatology or “sickness-behavior syndrome” (i.e., reduced saccharin preference and consumption, decreased body weight, decreased classical conditioning). In the second study (Müller et al., 2015), mice treated with this antibody exhibited weight loss, decreased activity, and increased serum levels of TNF, IL-6, IL-10, IL-18, and IFN-γ. Taken together, these findings support the role of increased CD40 signaling in depression and inflammatory processes.

Findings from two preclinical studies suggest that antidepressant treatment may decrease expression of *Cd40* and other inflammatory markers (O’Sullivan et al., 2009; Ślusarczyk et al., 2018). Using a lipopolysaccharide (LPS)-induced model of inflammation, the effects of two noradrenaline reuptake inhibitors (i.e., atomoxetine, desipramine) on the expression of inflammatory genes in the cortex of rats were evaluated (O’Sullivan et al., 2009). Compared to controls, rats treated with LPS had increased cortical expression of *Cd40*, *Nfkb*, *Tnf*, and *Il1b*. In the rats treated with atomoxetine or desipramine prior to the administration of LPS, cortical expression of *Cd40*, *Nfkb, Tnf, Il1b*, and inducible nitric oxide synthase decreased. In another study (Ślusarczyk et al., 2018), the effect of tianeptine treatment on rat microglial cells stimulated with LPS was evaluated. While these microglial cells exhibited increased expression of *Cd40* compared to control cells, *Cd40* expression was moderated in cells treated with tianeptine. In addition, tianeptine treatment prevented the upregulation of *Tnf, Il1b, Il6*, and *Il18*.

These preclinical studies provide new insights into the mechanisms of action of antidepressants. In addition, they support the associations between LPS-induced inflammatory responses and depressive symptoms in humans (Dantzer et al., 2008). While we do not know if the patients in our psychological cluster group were on antidepressants,

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our findings are consistent with previous studies that suggest increased expression of *CD40* is associated with depressive symptoms (O'Sullivan et al., 2009; Ślusarczyk et al., 2018).

Limitations and Future Directions

Several limitations warrant consideration. First, given our study's cross-sectional design, future research needs to determine whether associations between psychological cluster group membership and methylation levels change over time. Second, because the two samples were heterogeneous regarding gender, cancer type, and sample sizes, confirmation of these findings is warranted. Third, given that we did not evaluate antidepressant use, future research needs to evaluate the effect of antidepressants on psychological symptom cluster group membership. Fourth, we could not use these scores as a continuous value due to the statistical challenges encountered with the distribution of the psychological cluster factor scores. Additional research is warranted to evaluate how to use symptom cluster factor scores in epigenetic analyses. Finally, this analysis examined only transcriptional regulatory mechanisms (i.e., total expression levels of *CD40*) and not post-transcriptional regulatory mechanisms (e.g., alternative splicing). Future research is needed to evaluate the role of the splice variants of *CD40* in developing a psychological cluster.

Conclusion

This study is the first to evaluate for epigenetic regulation of inflammatory processes that underlie a psychological cluster in patients receiving chemotherapy. Our findings provide new evidence to support the hypothesis that inflammatory processes underlie the occurrence of a psychological cluster in these patients. By using a rank aggregation method to identify genes across two samples, multiple lines of evidence were integrated to identify the role of *CD40* in the occurrence of the psychological cluster. These findings provide preliminary evidence to suggest that epigenetic regulation of *CD40* may be involved in the occurrence of a psychological symptom cluster and suggest a direction for mechanistic studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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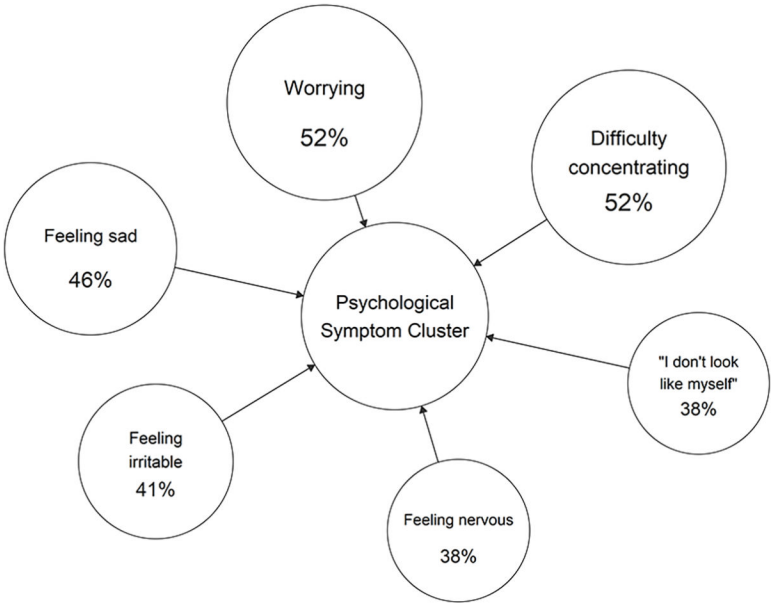

Figure 1.

Symptoms within the psychological 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).

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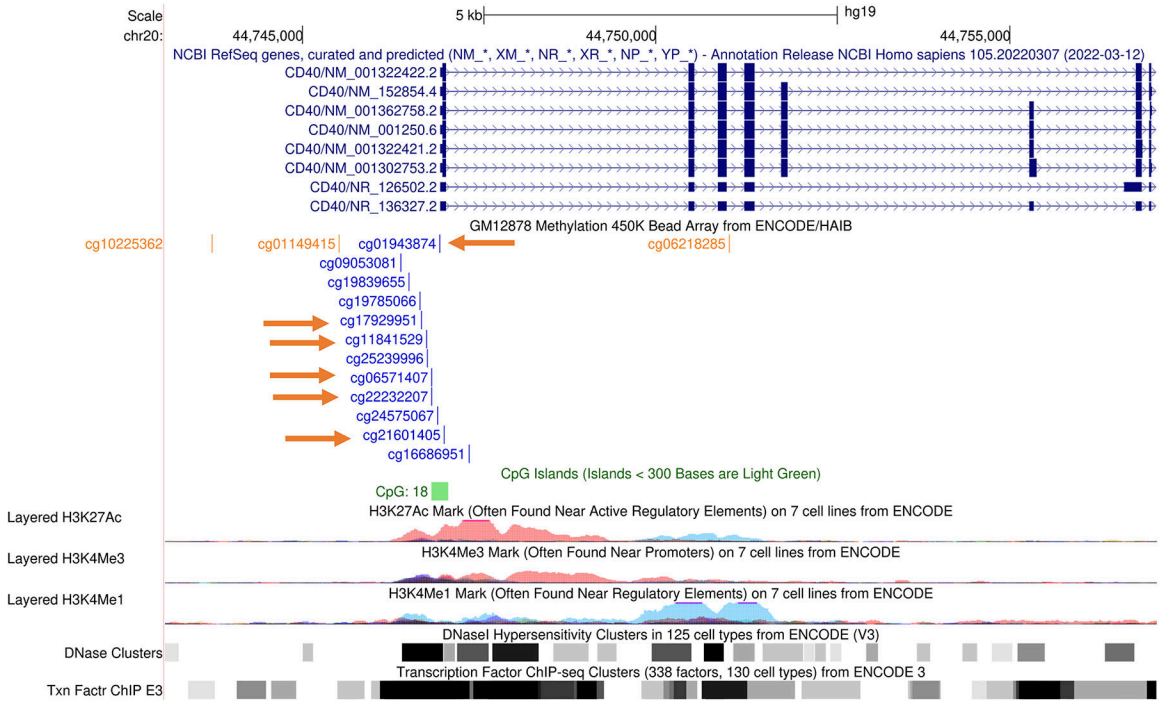

Figure 2.

Screenshot of the University of California Santa Cruz Genome browser (http://genome.ucsc.edu/) displaying the promoter region of CD40 (i.e., 2500 bp upstream and downstream of the transcription start site) on chromosome 20 of the hg19 (genome reference consortium Version 37) assembly of the human genome (Kent et al., 2002). Assembly tracks show scale, chromosome, the genomic position of the six eCpG loci associated with the psychological cluster (orange arrows), and their unmethylated status as reported by the HAIB. The CD40 gene models are provided by the NCBI RefSeq. The gene models depict exons as solid blocks connected by lines in introns with arrows showing the direction of transcription. Tracks denoting putative regulatory regions identified by ENCODE (Rosenbloom et al., 2013) include: a CpG island (i.e., 5'-C-phosphate-G-3' linear DNA sequence); levels of enrichment for the layered H3K27Ac, H3K4Me3, and H3K4Me1 histone marks; DNase I hypersensitivity clusters; and transcription factor ChIP-seq clusters. For the H3K27Ac, H3K4Me3, and H3K4Me1 marks, the coloring indicates a different signal intensity from one of seven cell lines. For the DNase I hypersensitivity and transcription factor ChIP-seq clusters, the darkness of the shading corresponds to the strength of the signal intensity indicating the presence of cis-regulatory elements or transcription factors.

Note. bp = base pairs; CD40 = cluster of differentiation 40; ChIP-seq = chromatin immunoprecipitation sequencing; chr = chromosome; eCpG = expression-associated CpG; ENCODE = Encyclopedia of DNA elements; GM12878 = B-lymphoblastoid cell line; H3K4me1 = histone H3 lysine 4 mono-methylation; H3K4me3 = histone H3 lysine 4 trimethylation; H3K27Ac = histone H3 lysine 27 acetylation; HAIB = Hudson Alpha Institute for Biotechnology; hg = human genome; RefSeq = National Center for Biotechnology Information Reference Sequence

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Table 1

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 to 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
Childcare 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

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$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
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
Psychological symptom cluster factor score distribution		
0	23	15.8
1	24	16.4
2	14	9.6
3	10	6.8
4	21	14.4
5	26	17.8
6	28	19.2

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

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Table 2

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 to 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
Childcare 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		

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< $30,000	142	17.3
$30,000 to < $70,000	172	20.9
$70,000 to < $100,000	143	17.4
≥ $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	66.4
Type of cancer		
Breast	365	39.5
Gastrointestinal	314	34.0
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
Psychological symptom cluster factor score distribution		
0	184	19.9
1	169	18.3
2	121	13.1
3	124	13.4
4	131	14.2
5	103	11.2
6	91	9.9

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

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Table 3

Five Highest Ranked Inflammation-Related Genes Using the Robust Rank Aggregation Method

Rank	Gene symbol[a]	Gene name[b]	Rank 450K	Rank EPIC	FDR[c]
1	*CD40*	Cluster of Differentiation 40 molecule	1	1	0.017
2	*PPP3CC*	Protein Phosphatase 3 Catalytic Subunit Gamma	19	2	1.000
3	*CAT*	Catalase	2	51	1.000
4	*IRF5*	Interferon Regulatory Factor 5	23	4	1.000
5	*PRF1*	Perforin 1	24	20	1.000

Note. FDR = false discovery rate

[a] HUGO Gene Nomenclature Committee-approved symbol

[b] HUGO Gene Nomenclature Committee-approved name

[c] Benjamini-Hochberg procedure